1985 Annual Report

Biological Control of Weeds Laboratory - Europe, USDA-ARS

Rome, Italy

BIOLOGICAL CONTROL OF WEEDS LAB PERSONNEL

Paul H. Dunn	Research Entomologist, Location Leader
Stephen L. Clement	Research Entomologist
Antonio Rizza	Research Entomologist - retired March, 1985
Pasquale Pecora	Research Entomologist
Luca Fornasari	Research Entomologist - EOD 12/1/85
Gaetano Campobasso	Agricultural Research Assistant
Tiziana Mimmocchi	Agricultural Research Clerk
Massimo Cristofaro	Agricultural Research Clerk
Massimo Stazi	Agricultural Research Clerk
Donatella Magni	Administrative Clerk - Transferred 10/1/85
Claudine Vincenti	Administrative Clerk - EOD 10/1/85
Antonio Laregina	Gardener/Maintenance
Rouhollah Sobhian	Research Entomologist, Univ. of Calif.
Antonio Taricone	Contract Researcher, Thessaloniki, Greece.
Carla Marangoni	Part-time Maintenance Man
	PIT Summer Laboratory Assistance

Cover photograph

By Massimo Cristofaro

Simyra dentinosa
Lepidoptera, Noctuidae

These moth larvae seriously defoliate *Euphorbia* plants so this species is being screened as a candidate for the biological control of leafy spurge.

NOT FOR PUBLICATION

NOTICE

The results of this report are preliminary and should not be quoted or discussed in publications without permission of the responsible scientist. If there is need to refer to this work, please correspond with the scientist and include a copy of the pertinent portion of your manuscript. The work should be cited as a personal communication and not in the bibliography. This report has an extremely limited distribution and is intended only to provide a means of communication among scientists and to provide a historical record of our laboratory.

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INTRODUCTION

1985 was an eventful year for the Rome laboratory both in the goals accomplished and the change of personnel.

Two insects made it through screening and evaluation and were liberated in the U.S. The seedhead weevil Bangasternus orientalis was liberated against yellow starthistle and the gall midge Bayeria capitigena was released on leafy spurge. A second gall midge Dasineura capsulae was cleared to be introduced into quarantine and the host specificity testing results of the weevil Bangasternus fausti (for diffuse knapweed) were favorable. Collections of previously introduced insects were made to provide additional individuals for release in new areas of the U.S., and to enrich the gene pool of the species already released and established.

In addition to these accomplishments, taxonomic revisions of the genera Apion and Bangasternus financed by extramural grant were complete enough to provide us with correct names for the insects with which we were working.

A major change in the laboratory staffing occurred when Antonio Rizza, our senior Italian scientist retired. Mr. Rizza had been with the laboratory for 23 years. In December Dott. Luca Fornasari (University of Pisa) was selected from an extensive list of candidates to fill the vacancy left by Mr. Rizza. Some time later, Mrs. Donatella Magni, who had been Administrative Clerk for about 10 years found employment at the Embassy, within walking distance of her home. Mrs. Magni's leaving could have been crippling to the laboratory but

we were most fortunate to be able to convince Mrs. Claudine Vincenti to come from Paris to fill the Administrative Clerk position. Mrs. Vincenti brought with her some 28+ years of experience in ARS, so the transition was not detectably disruptive.

As Research Leader, I was pleased with the results of our team's work in 1985 as well as the plans made and work started for 1986.

Paul H. Dunn,

Research Leader.

A petition for the introduction of the gall midge Dasineura capsulae Kieffer (Diptera: Cecidomyiidae), into quarantine for further testing. A candidate for the biological control of leafy spurge

Prepared by
P. Pecora, M. Cristofaro and M. Stazi

USDA/ARS, Biological Control of Weeds Laboratory-Europe
American Embassy, AGR
APO New York, N.Y. 09794-0007

Flower bud galls caused by D. capsulae larvae on E. esula

SYNOPSIS

A gall midge Dasineura capsulae, whose larvae cause flower bud galls on Euphorbia spp., and prevent seed production, was selected as candidate agent for the biological control of leafy spurge (Euphorbia esula-virgata "complex"), in North America. Studies of a population of D. capsulae at S. Rossore (Pisa, Italy), indicated that this midge has one generation per year, that the adults usually appeared in early April, and gradually continued to emerge from the soil until late May. Females laid eggs in the inner part of the bracts which cover the cyathium and the neonate larvae moved into the small cup-shaped cyathium after hatching. Galls, which first appeared in early May, were produced by the enlargement and distortion of the cyathium, thus stopping the seed production in the attacked flowers. The larvae of D. capsulae required ca. 4 weeks to complete their development and at the end of June and early July, left the galls, fell to the ground where they burrowed beneath the soil and hibernated until the following spring, pupating a few days before the adults emerged.

To determine the host-range, D. capsulae was tested in the laboratory and field with 49 plant species or varieties in 16 families and the midge oviposited in only 13 test plants and controls (all in the genus Euphorbia). Oviposition occurred on 10 test-plants in the subgenus Esula, and one test plant in each of the subgenera Agaloma, Poinsettia and Euphorbium. On E. peplus, E. helioscopia, E. milii, and E. pulcherima eggs were laid only occasionally. D. capsulae completed its development only on six of the test-plants on which it oviposited and they were all in the subgenus Esula. Field trials showed that North American biotypes of leafy spurge were suitable hosts for D. capsulae.

The restricted host-range of D. capsulae suggests it would be safe to use as a biological control agent against leafy spurge in North America. The safety of the midge is also suggested by its failure to develop on E. supina, E. maculata and E. serphyllifolia, three species which are broadly sympatric with leafy spurge (potential bridging species). The presence of this midge will complement the action of another recently introduced midge, Bayeria capitigena, whose larvae damage the meristematic tips. The two midges will cause more stress on the plant and act to limit its seed production and vigor, hence its spread.

I. Introduction

Leafy spurge, Euphorbia esula-virgata "complex" (Euphorbiaceae), is a weed of European origin that has become a serious problem in pastures, ranges and non-cropland areas in North America.

The taxonomic status of this "complex" is particularly confused. For example in a study based on a limited sampling of members of the "esula-aggregate", adventive in North America, Dunn and Radcliffe-Smith (1980) recognized 5 entities: (a) Euphorbia esula L. s. str.; (b) Euphorbia esula L. s. l. (E. androsaemifolia Willd.); (c) Euphorbia virgata Waldst. and Kit. var. uralensis (Fisch. ex Link) Boiss; (d) Euphorbia virgata Waldst. and Kit. var. orientalis Boiss. (E. boisseriana (Woron.) Prohk.); and (e) Euphorbia pseudovirgata (Schur.) Soo (E. esula L. x E. virgata Waldst. and Kit.) (E. intercedens Podp., non Pax) (E. podperae Croizat). However, in a recent detailed study, Radcliffe-Smith (1985) recognized a total of 11 species and 10 hybrids as members of the "esula-aggregate" naturalized in North America. This weed becomes an irreversible, dominant species on rangelands and pastures, displacing useful forage plants. It is also a poisonous plant producing an irritant that causes dermatitis to men and animals (Kingsburg 1964), and cattle usually refuse leafy spurge as food unless it is given to them in weedy hay or better forage is not available. According to Dunn (1979), leafy spurge occurs in 25 states with 451 infested counties. A conservative estimate of loss in the U.S., in terms of expenditure for controlling leafy spurge and lost of productivity, is $10.5 million annually (Noble et al. 1979).

The area of worst infestation in North America is defined by a 1,200 mile-diameter circle, centered near Wolf Point, Montana. This area covers parts of 9 states and 5 Canadian provinces and encompasses nearly 2.5 million acres. In the U.S., Minnesota has the highest infestation (800,000 acres), followed by North Dakota and Montana with 600,000 and 543,000 acres, respectively (Noble et al. 1979). The problem is most severe on undisturbed lands, but on cultivated cropland areas where leafy spurge has been controlled, it can reduce crop yields from 10 to 100% (Derscheid and Wrage, 1972).

This alien weed is continuing to spread at an almarming rate. The weed is difficult and expensive to control by cultural, mechanical, and chemical means, or combination of these methods.

Because of its foreign origin and the large number of natural enemies associated with it in Eurasia, leafy spurge is considered to be an excellent candidate weed for biological control.

A program for the biological control of leafy spurge was started by the U.S. Department of Agriculture in 1973. Efforts by the C.I.B.C., financed by Agriculture Canada, have resulted in the release of three species of insects (2 moths and 1 beetle) in North America. The spurge hawkmoth (Hyles euphorbiae L., Lepidoptera: Sphingidae), whose larvae defoliate spurge plants, was first released in the United States (Montana) in 1974 (Baker and Anderson 1974), and has recently become established on E. esula-virgata (R. Nowierski pers. comm.). From a later release, the moth became established in New York on E. cyparissias and on the hybrid E. x pseudo-esula Schur. (Batra 1983). The clearwing moth (Chamaesphecia tenthrediniformis, Den. & Shiff., Lepidoptera: Sesiidae), with root boring larvae, was first released in 1977 (P.H. Dunn pers. comm.), but did not become established, probably because it was too host specific to develop on the American biotypes of leafy spurge.

A longhorned beetle (Oberea erythrocephala Schrank, Coleoptera: Cerambycidae) has been periodically released between 1980 and 1984. To avoid the problem of hyperspecificity experienced with the clearwing moth, Schroeder (1980) tested O. erythrocephala on U.S. biotypes of leafy spurge during the initial phase of his research with this beetle and found the beetle would accept them. As a result Oberea has become established in Montana (N.E. Rees pers. comm.). In addition a flea beetle, (Aphthona flava Guill., Coleptera: Chrysomelidae) cleared by Canadian and CIBC workers and a gall midge (Bayeria capitigena (Bremi), Diptera: Cecidomyiidae), screened by the USDA Rome laboratory, were both released in 1985.

In order to provide additional biotic agents for the biological control of leafy spurge, we focused our study on a gall midge, Dasineura capsulae Kieffer, because a heavy infestation of this midge will reduce the seed production of leafy spurge. To determine the safety and effectiveness of D. capsulae as biocontrol agent, host specificity tests were conducted both in the field, at S. Rossore (Pisa), and in the laboratory, at the USDA facility at Rome, from 1982-1985.

II. Taxonomic position

The genus Dasineura (Rondani, 1840) is in the family Cecidomyiidae (Diptera), subfamily Cecidomyiinae, supertribe Oligotrophidi, and tribe Dasineurini (Ruebsaamen and Hedicke 1925-39). The species was described by Kieffer (1901) as Dasineura capsulae, but later he (Kieffer 1913) transferred it to the genus Perrissia, then Ruebsaamen and Hedicke (1925-39), returned the species capsulae to the genus Dasineura.

The genus Dasineura is comprised of about 300 species. Buhr (1964) lists 205 species distributed in Northern and Central Europe and Stone et al (1965) reported 95 species of Dasineura in America North of Mexico.

III. Geographic distribution

D. capsulae is widely distributed in Europe (Kieffer 1898). We have found this midge in Italy, Austria, Hungary, Romania and Bulgaria during our surveys for natural enemies of leafy spurge.

IV. Host plants

D. capsulae is known only from Euphorbia spp. Kieffer (1901) and Houard (1908-1909-1913) gave the following species as host plants: E. cyparissias L., E. esula L., E. nicaeensis All and E. phytusa L. In addition Buhr (1964) reported the midge from E. falcata L., E. lucida Waldst. & Kit., E. palustris L., and E. virgata Waldst. & Kit.

V. Life History

Material and Methods: In order to determine the oviposition period of D. capsulae and the phenology of the galls produced by this midge, periodic inspections were made at S. Rossore (Pisa, Italy) on April 2, April 19, May 2, May 26, June 15 and July 10, 1983. At each inspection, depending on which stage was available, a sample of 100 flower buds or 50 galls of various sizes were randomly collected from 10 plants of E. esula. In the inspections made in April only flower bud samples were collected. In the May and June inspections both flower bud and gall samples were taken, while in July only galls were collected. Buds and galls were dissected under a dissecting microscope and eggs, living and dead larvae, and parasites of the gall midge were recorded. A sample of 25 mature galls, collected on July 10, were measured in width and length. These data were treated by analysis of variance (ANOVA) and the means were separated by a Student-Newman-Keuls (a posteriori) test (Sokal and Rohlf, 1969).

To provide biological data of D. capsulae, 200 bud galls containing larvae of various stages were collected on E. esula at S. Rossore (Pisa, Italy), on June 15, 1982. These galls were brought to the laboratory and stored in a refrigerator in a closed polyethylene bag (Temp. 4^{o}-6^{o}C) for 4-5 days. The low temperature and high humidity, allowed to the mature larvae of D. capsulae to leave the galls. Using a fine brush, these mature larvae were transferred to 4500-ml acrylic containers with a 2-cm deep layer of moistened peat moss and a fine sand mixture on the bottom which provided a suitable substrate for the diapusing insects. Each box was covered by a plastic lid which had a 2 cm diameter central hole plugged with cotton, to allow some air exchange. Three containers, each with 200 mature larvae of D. capsulae were prepared and held undisturbed in an outdoor insectary until adult emergence started. The containers were checked daily and the emerged adults of D. capsulae and associated parasites were collected and recorded. Also the number of adults emerging from 1150 and 9000 mature larvae collected in June 1983 and 1984 from E. esula galls at S. Rossore were counted and recorded.

Some of the adults which emerged in 1983 were used to get oviposition and larval behavior data under laboratory conditions. To do this, the newly emerged adults, collected by a mouth aspirator, were placed in 300-ml acrylic plastic cages with the tops covered with nylon screen and the bottoms fitted snugly over 1.5-2 cm thick cork or balsa wood disks that had central holes. E. esula flower buds on growing plants, (6-8/cage) were then passed through these holes, thus allowing the insects to be caged directly on the plant. The cages were supported on the plants by fastening them with masking tape to metal rods inserted in the soil of the potted plant. To conduct these studies 8 cages were prepared with 2♀♀ 1♂/cage. Once the adults were exposed to the host plant, they were left undisturbed until they died, then the cages were

removed and the buds which had been exposed to the midges were marked. During this trial, 56 E. esula flower-buds were offered to the adults and after the cages were removed, sixteen buds (2 buds from each cage) were selected at random and checked for eggs. Another stock of 16 flower-buds (2 buds/cage) were dissected a week later and the behavior of the newly hatched larvae was observed. The remaining infested flower buds were left undisturbed until mature galls were formed; then all the galls were dissected and the number of living and dead larvae found in each gall was recorded.

To determine adult longevity and egg production per female, another 13 cages were prepared. In each cage, 3-4 flower buds of E. esula were exposed to a male and a female midge. The cages were inspected daily and the number of dead insects present were collected and recorded. When all the adults had died the flower buds were dissected and the number of eggs found on each was counted and recorded. The percentage egg fertility and the pre-eclosion period were determined from a sample of 507 newly laid eggs, kept in 128-ml plastic hatching containers (plastic cups which had a layer (2 cm thick) of moist plaster of Paris on the bottom and were closed by plastic caps).

The development time from neonate to mature larva was determined by preparing 18 300-ml acrylic cages, and exposing 15-20 flower buds to adults of D. capsulae ($3\varphi\varphi$ $2\sigma\sigma$/cage). These studies which started on April 20 and terminated on July 10 were done in a laboratory room with a constant temperature ($20^{\circ}C \pm 2^{\circ}$) and natural lighting and photoperiod. The exposed flower buds in 3 of the cages were dissected every week, the number of galls formed were recorded, and the larvae collected were preserved in 70% alcohol and later measured in length and width. The mean length of these larvae collected in the various weeks, were separated by a t test.

Biometric data of all stages of D. capsulae were taken. The width of the preserved larvae was taken at the widest part, while the width of the pupae and adults was taken on the first abdominal segment. The adult length measurements were exclusive of antennae and ovipositor.

Results

The periodic dissection of bud and gall samples collected at S. Rossore to investigate the life cycle of D. capsulae, yielded the following results:

In April only buds were present on the plants, and the mean number of eggs/bud laid on buds sampled April 19 and May 2 was not significantly ($P > 0.05$) different, whereas a significantly ($P < 0.05$) fewer number of eggs/bud were found on the sample of buds collected May 11. The first galls were found on May 2, but the majority (ca. 80%) of them were still in an early stage of development. On July 10 only mature galls were found and they had a mean length of 8.36 ± 1.51 mm (range = 5.60 - 12.40 mm) and mean width of 5.06 ± 0.95 mm (range = 3.68 - 6.80 mm) ($n=25$). The mean number of larvae found per gall was not significantly ($P > 0.05$) different between the various samples collected in May. A significantly ($P < 0.05$) lower number of larvae was found on the samples taken in June and July. In a sample of 50 galls taken on July 10, half of them were empty, 17 contained both mature larvae of D. capsulae and parasite pupae and 7 had only D. capsulae larvae; in one gall a single parasite adult was found. The results of these dissections are presented in Table 1.

The field and laboratory results suggest that the population of D. capsulae from S. Rossore is univoltine. Since the first galls appeared at the beginning of May and the infested flower buds required 3-4 weeks to be transformed into mature galls, we assume that the first adults appeared in early April, and that they gradually emerged from the soil until late May. Mature galls occured in the field until the end of June. At that time, the mature larvae usually left the galls in conditions of high humidity, i.e. early in the morning or just after rain, falling to the ground where they burrowed beneath the soil and hibernated until the following spring, pupating a few days before the adults emerged.

From 600 mature D. capsulae larvae collected from E. esula in mid-June 1982 and held in the laboratory, 177 adults (106 ♀♀ and 71 ♂♂) or 19.5% emerged. The first adults appeared on May 2, 1983 and the emergence continued until May 23. In addition, 193 endoparasites (Inostemma sp. Hymenoptera: Platygasteridae)[1/] emerged, which killed 32.1% of D. capsulae larvae. The remaining larvae of this midge (48.4) may have been killed by an ectoparasite, Pseudotorymus sp. (Hymenoptera: Torymidae)[2/] or died for unknown reasons. Thirty seven Pseudotorymus adults emerged, but it is not clear how many of the dead larvae were killed by this parasite.

From 1,150 mature midge larvae collected on June 20, 1983 and held over the winter, 176 adults (96 ♀♀ 80 ♂♂) emerged (15.3% survival) between April 10 and May 10 1984. The larval mortality due to Inostemma parasitism was 41.3% (475 Inostemma sp. adults emerged). The rest of the larval mortality (43.4%) was due to the ectoparasite (Pseudotorymus sp. 82 adults emerged) and of unknown causes.

1/ Identified by P.M. Marsh, Systematic Entomology Laboratory, USDA-ARS

2/ Identified by E.E. Grissell, Systematic Entomology Laboratory, USDA-ARS

From 9000 larvae collected on June 24, 1984 and held over winter, the adult emergence started on April 9, 1985 and ended on May 15. A total of 1906 adult midges (1111 ♀♀ 795 ♂♂) or 21% emerged, also, 1746 adults of the endoparasite Inostemma sp. which caused 19.4% midge larval mortality emerged. In addition 311 adults of Pseudotorymus sp. emerged. The percentage mortality due to this ectoparasite and other unknown factors was 59.5%. The data from the 1984 collections are presented in Fig. 1.

Observations on the adult and larval behavior of D. capsulae were made in the laboratory in May 1983 using mature larvae collected in the summer of 1982 and the adults which they produced. When they were ready, the overwintering larvae moved to the surface of the substrate and transformed into pupae measuring 2.04 ± 0.18 mm long by 0.59 ± 0.03 mm wide (n=10), which were light red except for the undeveloped wings and legs which were reddish brown. When fully developed the adult split the pupal skin and emerged. Mating and oviposition usually occurred the same day of emergence. Caged females visited 3-5 flower buds before selecting one for oviposition. The time necessary to lay a group of eggs in a bud ranged from 15 to 25 minutes. Once a suitable flower bud was selected, the female extended her ovipositor and usually inserted it between the two bracts which cover the cyathium, laying her eggs between the bracts and the cyathium. However, eggs were sometimes also laid inside the cyathium. Newly hatched larvae coming from eggs laid between the bracts and the cyathium, were found either between the bracts on the upper part of the cyathium, or inside the cyathium. These observations suggest that the neonate larvae move into the small cup-shaped cyathium after hatching. The mean fecundity for 13 females was 89.00±35.14 eggs (range = 21-144).

Sixteeen E. esula flower buds were dissected to discover the oviposition site of D. capsulae, and 8 of these were infested with an average of 48.12±19.26 eggs/bud (range = 20-75). In flower buds (n=16), dissected a week later, six of them were infested with 1st instar D. capsulae larvae (30.66 ± 9.52 larvae/bud; range = 15-40). From another 24 flower buds left undisturbed for three weeks, 10 galls were obtained. These galls contained a mean of 13.70 ± 7.55 larvae/gall (range = 4-30).

The freshly laid eggs were white, slightly elongate, with rounded ends, had a smooth translucent, soft chorion, and measured 0.27 ± 0.02 mm in length and 0.07 ± 0.01 mm in width (n=40). The eggs hatched in 3-5 days and 89% of a sample of 507 eggs were fertile. Female longevity was 3.07±0.64 days (n = 13), while the males (n = 13) lived 2.41±0.69 days. The adults had the following measurements: female 2.32 ± 0.09 mm in length and 0.41 ± 0.02 mm in width (n=10); male 1.69 ± 0.06 mm in length and 0.41 ± 0.02 mm in width (n=10).

The study conducted to determine the larval development of D. capsulae generated the following information: from 54 flower buds of E. esula dissected a week after the beginning of the experiment, 21 were infested with young larvae measuring 0.29 ± 0.03 mm in length and 0.077 ± 0.007 mm in width (n=95). By the end of second week, the bracts of the leafy spurge flower still covered the cyathium which showed a thickening of the walls.

Forty-eight flower buds (young galls) were dissected and 15 of them were infested by D. capsulae larvae (L = 0.33 ± 0.02 mm; W = 0.08 ± 0.03 mm; n=78). The length of these second week larvae was not significantly different ($P > 0.05$) from that of larvae found in the first week dissection. By the end of the third week it was possible to recognize the infested flower buds because the bracts had started to open and the cyathium had become enlarged and reddish. Sixteen of these flower buds (immature galls) were dissected and the larvae measured. These larvae (L = 0.72 ± 0.23 mm; W = 0.16 ± 0.03 mm; n=63) were significatly longer ($P < 0.05$) than the second week larvae. By the end of the fourth week, the bracts were almost completely opened and the well-formed galls were visible. Ten immature galls were dissected and the larvae collected (L = 1.36 ± 0.31 mm; W = 0.34 ± 0.09 mm; n=57) were significantly longer ($P < 0.05$) than the third week larvae. In addition, on the fourth week larvae, the anterior portion of the sternal spatula was visible through the larval integument. By the end of the fifth week, the bracts had dropped from the inflorescence and the galls were mature. Eighteen of these galls were dissected and the associated larvae (L = 3.10 ± 0.19 mm; W = 0.79 ± 0.09 mm; n=65) were significantly longer than those measured at the end of the fourth week and the sternal spatula becoming more visible and well formed. At the end of the sixth week, eight mature galls were dissected and the orange colored larvae were measured (L = 3.09 ± 0.35 mm, W = 0.79 ± 0.09 mm; n = 48). The length of these larvae was not significantly different ($P > 0.05$) from those measured the fifth week. These mature larvae were almost ready to leave the galls to hibernate into the soil until next spring.

VI. Mortality Factors

Two parasites, *Inostemma* sp. and *Pseudotorymus* sp., affected the population of *D. capsulae* at S. Rossore. In addition, some larvae died as a result of unknown mortality factors (i.e. wrong conditions for hibernation, disease, predation etc). The parasitoid *Inostemma* sp. females probably oviposit in the eggs or the newly hatched larvae of the midge because in the laboratory the majority of *Inostemma* adults emerged about a week earlier than the *D. capsulae* adults (Fig. 1-b), and eggs and young larvae of *D. capsulae* were present when the parasitoids were sexually mature.

Despite being parasitized, the midge larvae were able to complete their development to the 3rd instar. The first sign of parasitism was a change in color from orange to light pink, then the larval exoskeleton became transparent and an internal pink mass gradually shrank toward the posterior of the larva. Later the light yellow pupa of *Inostemma* sp. could be easily seen through the transparent exoskeleton of the midge larva as it gradually darkened and became black. To emerge, the adult of *Inostemma* made a hole in the midge larval exoskeleton.

Adults and pupae of the ectoparasite *Pseudotorymus* sp. as well as larvae parasitizing all instars of *D. capsulae* were found in the galls. It is not clear how many larvae of *D. capsulae* are needed for a *Pseudotorymus* larva to complete its development but if the larvae are small several are certainly required. In the laboratory, the peak emergence of *Pseudotorymus* sp. was at the end of the second decade of April (Fig. 1b).

VII. Effects of D. capsulae on host plants

The galls produced by D. capsulae are formed in several ways. Normally at S. Rossore they are produced by the enlargment and distortion of the cyathium, but occasionally they are created by the deformation of the bracts which cover the cyathium, or a deformation of the leaves of the meristematic tips. The major effect of D. capsulae on the host plant is to prevent flowering and thus reduce seed production. In order to quantify the effectiveness of this midge, flowers were collected from random plant samples at both S. Rossore (Italy) (E. esula) and near Alland (Austria) (E. virgata). These samples were usually taken when the majority of the galls reached their maturity. For each plant, the number of flowers and D. capsulae galls present at the time of collection were counted and recorded.

The sample of 20 plants of E. esula, examined on mid-June 1983, had a mean of 92.30± 36.57 flowers/plant plus 31.71± 22.06 galls/plant (23.4% infested flowers), while a second sample of 30 plants of E. esula, examined on June 10, 1984 had 69.64± 45.44 flowers/plant plus 13.00± 7.92 galls/plant (18.8% infested flowers).

On 35 plants of E. virgata, examined on June 22, 1983 a mean of 70.75± 23.39 flowers/plant and 8.97± 7.75 galls/plant were counted showing that 11.25% of the flowers were infested. On 28 plants of E. virgata, examined on June 18, 1984, a mean of 119.82± 62.12 flowers/plant plus 17.17± 11.01 galls/plant, showed that 12.5% of the flowers were infested.

VII. Potential control value

Using the system developed by Harris (1973) to determine the value of a candidate, we arrived at a score of 20. This score puts the agent in the category of moderately or partially effective, so its action should be complemented with the introduction of additional agents.

Effectiveness score of *D. capsulae*:

1.	Host specificity	1
2.	Direct damage inflicted	1
3.	Indirect damage inflicted	0
4.	Phenology of attack	2
5.	Number of generations	0
6.	Number of progeny/generation	0
7.	Extrinsic mortality factors	4
8.	Feeding behavior	2
9.	Compatibility	2
10.	Distribution	4
11.	Effectiveness	3
12.	Size	0
		20

IX. Host specificity tests

Test plants:

To determine the host plant range of *D. capsulae*, tests were conducted in 1984 and 1985 with 49 plant species or varieties in 17 families. Species closely related to *Euphorbia* (order Euphorbiales), plants in other orders of the superorder Rosidae, and plants attacked by other species of *Dasineura* were included in this test plant spectrum. Heywood's Flowering Plants of the World (1978) was used as a guide in constructing our host specificity test plant list.

Plants or seeds of U.S. biotypes of leafy spurge were provided by the USDA, ARS, Biological Control of Weeds Laboratory, Albany, California, and the remaining plants (or seeds) in the list were obtained from botanical gardens or commercial seed companies.

Multiple choice host suitability test (field)

Material and methods: The object of this experiment was to determine if, in a field situation, feral adults of *D. capsulae* would select any of the exposed test-plants as hosts. The experiment was conducted on a hunting preserve at S. Rossore, Pisa, Italy, where a population of this midge and its host (*E. esula*) occur naturally.

The experimental site was selected in June 1983 in an area with dense stands of *E. esula* plants. At this time ca. 50% of these plants had *D. capsulae* galls. The 500 sq.m. site (10 x 50 m) situated along a canal, was left undisturbed until April 16, 1984 when the experiment started. In the experimental area three plots each 4.00 m x 4.00 m were set up. The experimental design for each plot was a randomized complete block, consisting of 5 treatments (three test-plants, and control plants A and B) repeated five

times (total of 25 blocks). The following test-plants were included in the trial: Leafy spurge biotypes from Nebraska, Montana, Wyoming and Oregon, Euphorbia peplus, E. milii, E. characias, E. pulcherrima and Linum narbonense. The test-plants and the control (control A) plants were grown in 22 cm diameter plastic pots and were taken from Rome to the Pisa test site where the pots were buried in the ground with their tops at soil level in the naturally occurring leafy spurge infestation. The "Control A" consisted of E. esula plants from S. Rossore transplanted into the same size pots as the test plants, while "Control B" was composed of randomly selected E. esula plants growing naturally in the test site at the experimental area. The naturally ocurring plants of leafy spurge, except those which were to serve as control B were removed from the area.

The length of time the plants were kept in pots before use in the experiment was not uniform. The leafy spurge plants from Montana, Nebraska and Wyoming were kept in pots for six months prior to the experiment, while the plants from Oregon were in pots for 18 months, and the plants of Control A were in pots for 12 months prior to use. For the other test plants this period ranged between four and six months.

In order to follow the occurrence and development of the galls, weekly observations were made from mid-April to the end of May. At the beginning of June, when the galls had reached maturity, the number of plants present in each block, plus the number of flowers and galls/plant were recorded.

The data from this field experiment were treated by analysis of variance (ANOVA) and the means were separated by a Student-Newman-Keuls (a posteriori) test.

RESULTS:

The data obtained in the multiple choice test conducted at S. Rossore are summarized in Table 2. In plot No. 1, the mean number of flowers/plant of the Nebraska biotype was not significantly different ($P > 0.05$) when compared with the number of flowers of both controls (A and B). A significantly greater ($P < 0.05$) number of flowers occurred on E. peplus and E. characias. In addition the number of flowers on the plants in Control B (naturally occuring was significantly greater ($P < 0.05$) that those on the Control A (potted plants). Galls were found only on the two controls and it was noted that the number of galls produced per plant and the percentage of galled flowers/plant was significantly greater ($P < 0.05$) on the control B than on the control A.

In plot No. 2, the number of flowers/plant on the test plants, and in control A, was significantly ($P < 0.05$) smaller than the number of flower/plant in control B. Galls were produced by the midge on both Oregon and Wyoming biotypes, as well as on both controls. The number of the galled flowers/plant was significantly greater ($P < 0.05$) on control B plants than on control A plants; but there was no significant difference ($P > 0.05$) between the number of galls/plant on the potted plants of Control A and the potted Wyoming and Oregon biotypes. There were no significant differences ($P > 0.05$) between the percentages of galled flowers/plant on the Oregon biotype and Controls A and B. A significantly ($P < 0.05$) lower percentage of galled flowers was found on the Wyoming biotype.

In plot No. 3, the number of flowers/plant on E. pulcherrima (Poinsettia) was obviously significantly fewer ($P < 0.05$) than on the other test and control plants. Galls were found on the Montana biotype of E. esula-virgata and on controls A and B. The number of galls/plant was significantly higher ($P < 0.05$) on control B than on control A and Montana biotype plants. There were no significant differences ($P > 0.05$) in the percentage of galled flower per plant.

No choice oviposition and host suitability test (Laboratory trial)

Material and Methods: To determine the range of plants that D. capsulae would accept for oviposition and plants which would support the development of the midge, 49 plant species or varieties were included in a no choice oviposition and host suitability test (Table 3). In order to provide vigorous adults of D. capsulae to use in these laboratory trials, 300 galls, containing midge larvae of various stages, were collected on E. esula at S. Rossore on June 11, 1983. From these galls we obtained 2,082 larvae of D. capsulae which were divided into 10 groups, each of which was placed in a previously prepared plastic container. In the second decade of June 1983, 106 D. capsulae galls were collected near Alland (Austria) and another 96 were collected near Debrecen (Hungary) on E. virgata. Twohundred forty-eight D. capsulae larvae were collected from the Austrian galls and they were put in 3 plastic containers (ca. 80 larvae/container). The Hungarian galls produced 169 larvae, which were equally distributed in 2 containers. On June 23, 1984, another 1,275 galls were collected on E. esula at S. Rossore and from these 9,000 mature larvae were collected and divided among 20 plastic containers (450 larvae/container).

All these containers were held in an outdoor insectary and checked weekly until adult emergence was noticed, then they were checked daily.

Newly emerged adults were transferred onto the plants in the acrylic plastic cages described earlier. Because of shortage of freshly emerged adults, all the plants could not be tested simultaneously so they were divided into troups and each group was tested using insects emerged the same day. A range of 8-25 flower buds/cage were exposed to 4-6 adult midges and 3-4 replications were made for each test-plant, one cage serving as a replicate. The plants with cages containing the midges were held in a laboratory room where the ambient temperature ranged between 21°C-25°C and were left undisturbed until the midges died. Later, in order to determine if oviposition had occurred, we started dissecting flower buds until eggs of D. capsulae were found in 1 or 2 of them. Once we had ascertained that oviposition had occurred, the other flower buds were left undisturbed and we used them to follow gall development. The infested plants were kept in pots, and were transferred from the laboratory to the garden. To determine the percent of egg hatch, eggs found in the bud dissections were placed in plastic hatching containers. As eclosion occurred, the neonate larvae were counted and the number recorded.

In April and May 1984 and 1985 the galls produced by D. capsulae on the test-plants as well as on the controls were counted and dissected, and the number of living and dead midge larvae and parasites per gall were counted and recorded.

The adults of D. capsulae emerging in the spring of 1984 from the larvae collected in Italy, Austria and Hungary, were used to test different North American biotypes of leafy spurge, while the adults which emerged in the spring of 1985, from larvae collected at S. Rossore, were used to test the other plants in the list. The data from this test were subjected to analysis of variance (ANOVA) and means were separated by a Student-Newman-Keuls (a posteriori) test.

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RESULTS: On 49 plant species or varieties tested, D. capsulae collected at S. Rossore accepted the controls plus 13 taxa for oviposition, all in the genus Euphorbia and was able to complete larval development on the controls plus 6 of the test-plants on which oviposition occurred. The results are presented in table 4. Because of their different botanical characters, some of the exposed test-plants had different numbers of flower buds than control plants. The test-plants, E. marginata, E. dendroides, E. cyparissias, E. helioscopia, E. milii, E. terracina and E. pulcherrima had significantly fewer ($P < 0.05$) flower buds than the controls. On the other hand, the mean number of flower buds present on the various leafy spurge biotypes (Group I) were not significantly ($P > 0.05$) different from the Italian Euphorbia esula controls.

In Group I, the mean number of eggs laid on the control and the test plants was similar ($P > 0.05$). In Group II, there was no significant difference ($P > 0.05$) in the number of eggs laid on E. amygdaloides and E. marginata and the control; but a significantly higher ($P < 0.05$) number was laid on E. dendroides and E. lucida. In group III, significantly more ($P < 0.05$) eggs were laid on the control than on the test plants (E. cyparissias, E. helioscopia and E. peplus). In group IV, similar ($P > 0.05$) numbers of eggs were laid on the control and E. terracina; significantly fewer ($P < 0.05$) were laid on E. milii and E. pulcherima than on the control plants. There were no significant differences ($P > 0.05$) in the percentage of viable eggs laid on the different test and control plants in any of the groups.

The number of galls produced on the Italian control plant and the Montana and Wyoming leafy spurge biotypes in group I were not significantly different ($P > 0.05$), but no galls were found on the Nebraska biotype. The number of galls found on *E. dendroides*, *E. lucida*, *E. cyparissias*, and *E. terracina* was not significantly different ($P > 0.05$) from the number produced on control plants in the corresponding groups.

The percentage of infested flower buds on plants was determined by GP/FBL x 100, where GP = number of galls present, and FBL = flower buds left for gall development. Using this formula, there was no significant difference ($P > 0.05$) between the mean percentages on the test plants and the controls in groups I, II, and III.

In Group IV the percentages of galls produced on *E. terracina* was significantly ($P < 0.05$) greater than on the control plants. No significant differences were detected in the number of larvae found in the galls produced on the test and control plants in any of the Groups.

Both the Austrian and Hungarian biotypes of the midge laid eggs and galls developed on the Montana biotype of leafy spurge; however, both midge biotypes did not lay eggs on *E. peplus*. There were no Hungarian control plants available for the tests with the insects of Hungarian origin. The insects of Austrian origin laid eggs and caused normal galling of control plants from Austria. There was no significant difference ($P > 0.05$) between the Control and the Montana plants in the number of flower buds present, the number of eggs laid or the percent viability of the eggs. Compared with the control a significantly fewer ($P < 0.05$) number of galls and lower percent infestation occurred on the Montana biotype. No significant differences ($P > 0.05$) in the number of larvae per gall were found.

No choice host suitability test (Laboratory)

Material and Methods: Adults of D. capsulae used in this trial came from Austria, Hungary and Italy and only the test-plants on which oviposition occurred in the former trials were included. Even though Euphorbia lucida should have been tested, it was not included in this experiment because of a shortage of plants. The North American biotypes of leafy spurge were tested in April and May, 1984, and the other plants were tested in April and May, 1985.

After emergence, adults of D. capsulae, from Italy, Austria and Hungary, were placed in acrylic plastic cages (2♀♀ 2♂♂/cage), and because of a shortage of freshly emerged adults, the test-plants were grouped as in the previous experiment. Three replications were made for each species of test-plant. The potted test plants with the insects caged on them were kept in a laboratory room with natural light, and the ambient temperature between 20° C and 25° C. When the insects died (ca. 3-4 days), the cages were removed, and the plants which had been exposed to the midges were moved out of doors to a shaded area in the laboratory garden and left undisturbed but kept under observation for the formation of Dasineura induced galls. The number of galls which appeared and percentage of infested flower buds were recorded and these data were subjected to analysis of variance (ANOVA), and the Student-Newman-Keuls (a posteriori) test was used for separation of the means.

Results: The data obtained in no choice host suitability experiment are presented in Table 5. The Italian population of D. capsulae generated the following information: the mean number of exposed flower buds were not significantly different ($P > 0.05$) between the test-plants and their respective control plants in groups I, II, and IV, but in group III, compared with the control a significantly lower number ($P < 0.05$) of flower buds were present on E. pulcherrima, and a significantly greater ($P < 0.05$) number on E. terracina. In the group I, no galls were produced on the Nebraska leafy spurge biotype, but D. capsulae galls were found on both Montana and Wyoming leafy spurge biotypes, as well as on the control, and the number of galls produced per test-plant did not differ significantly ($P > 0.05$) from those produced on the control. In group II, there was no significant difference ($P > 0.05$) between the number of galls produced on E. dendroides and its control, and no galls were produced on E. marginata and E. amygdaloides. In Group III, the number of galls produced on E. cyparissias and E. terracina did not differ significantly ($P > 0.05$) from the number found on the control and there were no galls on E. pulcherrima. In Group IV, seventeen percent of the control flowers were galled but no galls were formed on E. helioscopia or E. peplus.

In general, neither the percentage of galls/plant nor the number of midge larvae/gall was significantly different ($P > 0.05$) between the test-plants on which gall development occurred and their respective control plants.

Also, the Austrian population of D. capsulae, tested with Montana and Wyoming biotypes of leafy spurge showed no significant difference ($P > 0.05$) between test plants and the controls in the number of flowers present, the number of galls produced, and the percent infestation thus confirming the results of the previous laboratory trial.

The D. capsulae from Hungary were tested with both Montana and Nebraska biotypes of leafy spurge but no control plants were available. The midge oviposited, caused galls and developed only on the Montana biotype.

Discussion

Our studies showed a close synchronization between the period of emergence of D. capsulae and the pre-flower stage of its host-plant, E. esula. At S. Rossore, where observations were conducted on a population of this midge, the critical period was usually from mid-April to the end of May.

Also, it was demonstrated, under field conditions, that D. capsulae selected and completed development on some North American biotypes of leafy spurge (Fig. 3-b, c). This is an important finding in case this midge is introduced as a biological control agent. Since North American biotypes of leafy spurge are considered to be exotic plants in Italy they are kept and grown in pots and never planted in the ground. As a result there is often a difference in the quality of plants grown in pots and those occurring spontaneously in the field. For example, there were significantly fewer galls found on the control and test plants grown in pots than on the control plants growing naturally at the experimental site. In various inspections, it was observed that potted plants of E. esula and of the North American biotypes of leafy spurge had some chlorotic leaves and that ca. 50% of the flower buds dropped off the plant before reaching maturity, while the plants of E. esula, growing naturally in the area were in good vegetative condition. The poor condition of the potted test and control plants could be because these plants had been kept in pots from 6-12 months without fertilization prior to use in

the experiments. We assume that the lower infestations recorded on potted plants was generally due to plant conditions. In the laboratory, we noticed that the larvae of D. capsulae rarely developed on flower buds in poor condition because in the absence of a good nutritional tissue the larval development could not be completed. The negative results obtained on the Nebraska bioptye of E. esula-virgata could also be due to the lack of suitability of this taxon for the larval development of this midge.

Observations made in the laboratory also indicated that females of this midge usually (ca. 95% of the cases) selected healthy and vigorous flower buds in the "right stage" with the bracts completely covering the cyathium for oviposition. Occasionally, however, eggs were found on weak flower buds or flower buds in the "wrong stage" on which the bracts had already opened.

In the laboratory, D. capsulae was tested with 49 plant species or varieties in 16 families and the midge oviposited on only 13 test-plants and the control (all in the genus Euphorbia). Oviposition occurred on 10 test-plants in the subgenus Esula, and on one test-plant in each of the subgenera Agaloma, Poinsettia and Euphorbium. On E. peplus, E. helioscopia and E. milii, and E. pulcherrima eggs were laid only occasionally. The number of eggs laid by D. capsulae on the other acceptable test-plants was not significantly different from the number laid on their controls. The few eggs oviposited on the subgenera other than esula, suggest that flower buds may have contained deterrent substances which limited the attraction to the plant for oviposition. Although the number of eggs laid on E. marginata did not significantly differ from those on the control, the midges selected the external part of the bracts for oviposition. This abnormal behavior of D. capsulae could be due to the fact that E. marginata possesses the proper

attractants for oviposition, but not appropriate flower buds. The flower buds of E. marginata had hairy bracts, while on those of the control plant were glabrous. This hairiness probably interfered with the act of D. capsulae inserting its oviposition between bracts.

These observations suggest that synchronization with flower buds in the "right stage" and the presence of an attractant are important for the host selection and oviposition by D. capsulae.

D. capsulae completed its development on only six of the plants on which it oviposited and they were all in the subgenus Esula (Fig. 3). Eggs laid on the other test-plants (E. marginata, E. pulcherrima, E. milii, E. peplus, E. helioscopia) were fertile but the larvae did not develop, probably because the flower buds of these plants were morphologically different from those of the normal host plant (control) and normal galls could not form, thus preventing the development of the midge larvae.

No larval development occurred on E. amygdaloides or the Nebraska biotype of leafy spurge, even though flower buds of these plants were apparently morphologically similar to those of the control. In these cases, the failure of larval development of D. capsulae may have been due to the presence of feeding deterrents or lack of essential nutrients, either of which make the taxa unsuitable.

The restricted host-range of D. capsulae suggests that it would be safe to use this insect as biological control agent of leafy spurge in North America. The safety of the midge is also suggested by its failure to develop on Euphorbia supina, E. maculata and E. serphyllifolia (Fig. 5-d, e, f), three species which are broadly sympatric with leafy spurge (potential bridging species).

At S. Rossore, where this midge is heavily parasitized, it is frequently able to infest ca. 20% of the exposed flower buds of E. esula. We believe that once released in the U.S., freed of its native parasitoids, the midge may have a greater potential for reduction of seed production of leafy spurge than it has in Europe where it is heavily parasitized. In addition the presence of this midge will complement the action of a recently released gall midge, Bayeria capitigena. The two midges will inflict more stress on the plant and act to limit its seed production and hence its spread.

Two populations of D. capsulae coming from Austria and Hungary have demonstrated their ability to develop on some of the North American biotypes of leafy spurge, therefore, other European biotypes from other places climatically homologous with U.S. and Canadian environment, can most likely be found and introduced.

If approval is granted for the introduction of this insect into quarantine at the USDA-ARS Biological Control of Weeds Quarantine Laboratory at Albany, California, the U.S. native and endangered species not tested in Rome will be tested there, prior to petitioning for release.

XI. Summary

The following points suggest that D. capsulae warrants serious consideration for approval for introduction into quarantine where additional host specificity tests will be conducted:

1) Literature host records indicate that D. capsulae is associated only with the genus Euphorbia.
2) There are no literature records of host plants of economic or social importance.

3) Based on host specificity studies in Rome, Italy D. capsulae has a host range restricted to the genus Euphorbia (subgenus Esula).

4) Most U.S. biotypes of leafy spurge tested were found to be suitable hosts.

5) Since the gall midge occurs over a wide climatic range, different ecotypes should be available for introduction into North America.

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Table 1. Periodic dissections of flower-buds and Dasineura capsulae galls collected on Euphorbia esula, at S. Rossore (Pisa), Italy.[a/]

	No.of infested flower-buds[1/] with eggs	No. of eggs/bud[2/] $\bar{X} \pm$ SD	Range	larvae/gall[3/] $\bar{X} \pm$ SD	Range	No. of parasites (Pseudotorymus sp.)[4/]	Number of empty galls
April 2	0	0		Galls not present			
April 19	12	29.42 ± 10.46a	17-50	0			
May 2	15	25.87 ± 12.81ab	8-45	13.56 ± 7.23a	4-35		
May 11	5	14.40 ± 6.31b	7-21	11.18 ± 5.56ab	3-30		
May 26	0			12.28 ± 6.40a	0-25		4
June 15	0			9.26 ± 7.57b	0-24	9	12
July 10	Buds not present	0		4.98 ± 6.27c	0-25	17	25

a/ Means in columns followed by the same letter are not significantly different ($P < 0.05$) according to a Student-Newman-Keuls (a posteriori) test

1/ Based on sample of 100 flower-buds.

2/ $\bar{x} \pm$ SD eggs/bud was calculated on the number of infested flower-buds.

3/ $\bar{x} \pm$ SD larvae/gall was calculated on a sample of 50 galls.

4/ Hymenoptera: Torymidae, an ectoparasite of D. capsulae larvae.

Table 2. Multiple Choice Host Suitability Test of *Dasineura capsulae*, conducted at S. Rossore (Italy), 1984[a/].

	No. of Plants [b/]	No. of flowers/plant $\bar{X} \pm$ SD	Range	No. of flowers galled/plant $\bar{X} \pm$ SD	Range	% of flowers galled/plant $\bar{X} \pm$ SD	range
PLOT 1							
Euphorbia esula Italy (Control A)	9	26.22 ± 14.92a	10–60	3.33 ± 2.95a	0– 6	12.53 ± 12.89a	0–37.50
E. esula Italy (Control B)	16	61.75 ± 44.17b	16–180	13.12 ± 11.05b	0–40	17.73 ± 9.28b	0–30.00
E. esula- virgata Nebraska biotype	6	34.00 ± 18.47ab	8–63	-		-	
E. peplus	5	209.80 ± 64.35c	164–320	-		-	
E. characias	5	110.80 ± 26.10d	81–150	-		-	
PLOT 2							
Euphorbia esula Italy (Control A)	11	33.63 ± 19.50a	10–75	5.18 ± 4.68a	0–17	15.96 ± 17.41a	0–62.96
E. esula Italy (Control B)	16	75.56 ± 43.26b	30–120	15.69 ± 9.08b	0–29	18.92 ± 10.63a	0–40.00
E. esula-virgata Oregon biotype	9	30.44 ± 13.76a	20–52	4.44 ± 9.26a	0–28	8.28 ± 13.26a	0–35.00
E. esula-virgata Wyoming biotype	9	29.22 ± 12.15a	20–60	2.00 ± 3.12a	0– 7	4.74 ± 7.30b	0–16.67
Linum narbonense	14	17.71 ± 7.06a	7–25	-		-	
PLOT 3							
Euphorbia esula Italy (Control A)	11	38.54 ± 14.91a	20–73	4.90 ± 4.86a	0–15	11.86 ± 11.63a	0–39.39
E. esula Italy (Control B)	12	63.83 ± 42.74a	25–175	13.75 ± 8.43b	0–26	18.39 ± 10.24a	0–34.21
E. esula-virgata Montana biotype	9	37.55 ± 27.28a	18–100	3.22 ± 3.96a	0–11	9.48 ± 11.87a	0–35.48
E. milii	5	77.20 ± 26.49a	35–103	-		-	
E. pulcherrima	8	8.90 ± 3.96b	6–12	-		-	

a/ Means in columns followed by the same letter are not significantly different ($P < 0.05$) according to a Student-Newman-Kleus (a posteriori) test.

b/ Control A: potted plants of *E. esula*; Control B: plants of *E. esula* naturally growing in the experimental area.

Table 3. List of plant species or varieties tested with Dasineura capsulae.

TEST PLANTS

1) Plants related to leafy spurge (Euphorbiaceae)

ORDER	SUBGENUS		SPECIES
Euphorbiales	Esula	**	Euphorbia esula L. - Italy (Control)
		*	E. esula-virgata - Nebraska biotype
		**	E. esula-virgata - Wyoming biotype
		**	E. esula-virgata - Montana biotype
		**	E. lucida Waldstein & Kitaibel
		**	E. terracina L.
		**	E. cyparissias L.
		*	E. amygdaloides L.
		**	E. dendroides L.
		*	E. helioscopia L.
			E. characias L.
			E. peplus L.
			E. lathyris L. cv. Chico
			E. lathyris L. cv. Castro Valley
	Agaloma	*	E. marginata Pursh
	Poinsettia	*	E. pulcherrima Willdenow
	Chamaesyce		E. maculata L.
			E. supina Rafinesque-Schmaltz
			E. serpyllifolia Persoon
	Euphorbium	*	E. milii Ch. des Moulins
			Ricinus communis L.
			Mercurialis annua L.

2) Plants attacked by other species of the genus Dasineura

ORDER	FAMILY	SPECIES
Geraniales	Linaceae	Linum narbonense L.
Rosales	Rosaceae	Rosa sp.
		Crataegus oxycantha L.
Capparales	Cruciferae	Alyssum saxatile L.
Fabales	Leguminosae	Trifolium incarnatum L.

3) Plants in other orders of the superorder Rosidae

ORDER	FAMILY	SPECIES
Rosales	Rosaceae	Potentilla fragiformis Willdenow
		Geum urbanum L.
Fabales	Leguminosae	Cytisus sp. L.
		Phaseoulus vulgaris L.
Myrtales	Onagraceae	Clarkia elegans Douglas
Sapindales	Rutaceae	Ruta graveolens L.
Geraniales	Geranaciaceae	Pelargonium zonale (L.) Aiton

4) Additional economic or ornamental plants which also provide superorder representation.

Ranuncolales	Ranuncolaceae	Anemone sp.
Caryophyllales	Caryophyllaceae	Dianthus sp.
		Cerastium tomentosum L.
Capparales	Cruciferae	Iberis sempervirens L.
Gentianales	Apocynaceae	Nerium oleander L.
		Vinca major L.
Lamiales	Verbenaceae	Verbena hybrida Voss
	Labiatae	Thymus serpyllum L.
Scrophuralialeles	Scrophulariaceae	Veronica teucrium L.
Asterales	Compositae	Tagetes glandulifera Schrank
		Centaurea cineraria L.
		Carthamus tinctorius L.
Commelinales	Gramineae	Hordeum vulgaris L.
		Triticum aestivum L.
Liliales	Liliaceae	Lilium sp. cv. Tabasco

* = Plants on which oviposition occurred

** = Plants on which oviposition and larval development occurred. For details see Tables 4 and 5

Table 4. No Choice Oviposition and Host Suitability Test of D. capsulae[a/]

TEST PLANTS	No. of Exposed Flower Buds	No. of Dissected Flower Buds	No. of eggs/bud	% of Viable Eggs [d/]	No. of Flower Buds Left for Gall Development	No. of galls	% infestation [e/]	No. of larvae/gall
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
			Dasineura ex. Euphorbia esula - Pisa (Italy)					
GROUP I[b/] Euphorbia esula Italy (Control)	26.25 ± 8.96a	5.25 ± 3.40	24.75 ± 17.61a	92.87 ± 1.11a	21.00 ± 8.41	7.50 ± 6.14a	32.91 ± 31.34a	5.30 ± 5.08a
E. esula-virgata Montana biotype	18.50 ± 6.85a	3.25 ± 1.89	24.50 ± 9.47a	88.74 ± 8.33a	15.25 ± 5.85	6.75 ± 2.50a	44.87 ± 3.84a	3.44 ± 3.47a
E. esula-virgata Wyoming biotype	19.75 ± 4.35a	5.25 ± 1.89	16.00 ± 7.07a	87.66 ± 5.15a	14.50 ± 5.74	6.00 ± 3.74a	38.39 ± 10.66a	4.04 ± 3.86a
E. esula-virgata Nebraska biotype	15.75 ± 3.86a	4.50 ± 1.91	17.80 ± 13.22a	91.83 ± 3.34a	11.25 ± 2.50	-	-	-
GROUP II[c/] Euphorbia esula Italy (Control)	44.50 ± 6.24a	24.50 ± 18.01	6.75 ± 6.70a	90.28 ± 8.67a	19.75 ± 23.42	6.25 ± 9.46a	35.74 ± 35.17ab	5.24 ± 5.13a
E. amygdaloides	38.25 ± 15.06a	12.50 ± 5.26	17.75 ± 11.90ab	93.83 ± 4.65a	25.75 ± 20.04	-	-	-
E. marginata	9.00 ± 2.45b	4.00 ± 2.16	12.00 ± 15.68ab	92.02 ± 6.93a	5.00 ± 3.56	-	-	-
E. dendroides	15.50 ± 5.97b	2.50 ± 1.00	31.40 ± 11.67b	94.02 ± 4.78a	13.00 ± 5.77	2.00 ± 2.45a	12.50 ± 14.43a	4.37 ± 3.50a
E. lucida	37.50 ± 6.76a	13.50 ± 11.47	29.50 ± 16.78b	96.35 ± 4.97a	24.00 ± 17.79	12.25 ± 8.34a	54.53 ± 19.71b	4.31 ± 3.00a
GROUP III[c/] Euphorbia esula Italy (Control)	49.75 ± 9.67a	12.00 ± 9.38	28.20 ± 6.38a	94.09 ± 4.11a	37.75 ± 3.30	6.25 ± 4.35a	15.94 ± 10.82a	4.08 ± 3.20a
E. cyparissias	21.00 ± 9.56b	8.25 ± 6.13	9.25 ± 5.74b	95.83 ± 8.33a	12.75 ± 9.67	3.75 ± 4.50a	17.68 ± 20.64a	3.20 ± 3.40a
E. helioscopia	20.00 ± 12.25b	4.25 ± 4.27	3.75 ± 6.85b	96.43 ± 5.05a	3.25 ± 4.27	-	-	-
E. peplus	32.50 ± 10.38ab	16.25 ± 12.60	6.00 ± 7.35b	93.33 ± 9.42a	16.25 ± 19.74	-	-	-
GROUP IV[c/] Euphorbia esula Italy (Control)	31.00 ± 2.58a	5.25 ± 3.95	12.50 ± 4.12a	98.61 ± 2.78a	25.75 ± 6.13	7.50 ± 5.80a	25.77 ± 19.13a	5.87 ± 4.97a
E. silii	8.75 ± 4.99b	5.75 ± 1.71	2.00 ± 4.00b	98.20 ± 4.50a	3.00 ± 6.00	-	-	-
E. terracina	13.50 ± 11.36b	4.00 ± 2.31	11.80 ± 17.58ab	97.62 ± 3.36a	6.75 ± 8.99	6.75 ± 8.99a	73.93 ± 8.58b	2.81 ± 2.92a
E. pulcherrima	14.50 ± 3.41b	10.50 ± 6.19	1.50 ± 3.00b	83.33 ± 3.47a	4.00 ± 8.00	-	-	-
			Dasineura ex. Euphorbia virgata - Alland (Austria)					
GROUP V[b/] E. esula-virgata Austria (Control)	15.50 ± 0.71a	4.00 ± 1.41	20.60 ± 11.99a	88.24 ± 9.23a	11.50 ± 2.12	7.50 ± 0.71a	66.92 ± 18.49a	4.37 ± 3.00a
E. esula-virgata Montana biotype	12.00 ± 2.00a	8.33 ± 4.73	21.75 ± 15.13a	92.42 ± 6.31a	3.67 ± 6.35	2.67 ± 4.62b	24.24 ± 41.99b	5.30 ± 3.47a
E. peplus	29.00 ± 26.87b	29.00 ± 26.87	0	-	0	0	-	-
			Dasineura ex Euphorbia virgata - Debrecen (Hungary) [f/]					
GROUP VI[b/] E. esula-virgata Montana biotype	10	3	9	100	7	2	28.57	5.60 ± 2.90
E. peplus	7	7	0	-	0	0	-	-

a/ Means followed by the same letter within a column are not significantly different ($P < 0.05$; Student-Newman-Keuls (a posteriori) test); $\bar{X} \pm SD$ (n=4)

b/ Plants tested in 1984

c/ Plants tested in 1985

d/ % of viable eggs = $\frac{\text{N. of hatched eggs}}{\text{N. of eggs}} \times 100$

e/ % of infestation = GP:FBL x 100 (GP = Galls present; FBL = flower buds left for gall development)

f/ Plant control not available; a replicate was made.

Table 5. No Choice Host Suitability Test of D. capsulae a/

TEST PLANTS	No. of Flower Buds $\bar{X} \pm SD$	N. of Galls $\bar{X} \pm SD$	% of Infestation d/ $\bar{X} \pm SD$	No. of larvae/gall $\bar{X} \pm SD$
		Dasineura ex. Euphorbia esula - Pisa (Italy)		
GROUP I b/				
Euphorbia esula Italy (Control)	26.33 ± 10.97a	4.67 ± 4.51a	14.76 ± 15.00a	4.21 ± 7.69a
E. esula-virgata Montana biotype	19.00 ± 4.00a	11.00 ± 9.85a	53.62 ± 50.39a	4.61 ± 8.05a
E. esula-virgata Wyoming biotype	23.33 ± 8.33a	9.67 ± 12.66a	30.51 ± 43.24a	4.24 ± 5.78a
E. esula-virgata Nebraska biotype	12.33 ± 2.08a			
GROUP II c/				
Euphorbia esula Italy (Control)	24.67 ± 11.01ab	7.33 ± 5.51a	26.87 ± 12.36a	5.23 ± 4.17a
E. amygdaloides	21.67 ± 4.04a	0	0	0
E. marginata	16.67 ± 2.08b	0	0	0
E. dendroides	24.00 ± 4.00b	2.67 ± 0.58a	11.07 ± 1.29a	2.87 ± 1.81a
GROUP III c/				
Euphorbia esula Italy (Control)	20.33 ± 3.78a	9.33 ± 5.86ab	22.02 ± 9.66a	3.93 ± 3.87a
E. cyparissias	18.00 ± 3.60a	3.00 ± 1.00a	17.33 ± 6.77a	3.44 ± 2.74a
E. terracina	31.67 ± 6.51b	10.67 ± 2.52b	34.40 ± 9.50a	2.56 ± 2.49a
E. pulcherrina	10.67 ± 2.31c	0	0	0
GROUP IV e/				
Euphorbia esula Italy (Control)	28.00 ± 2.64a	4.67 ± 0.58	16.64 ± 0.62a	4.43 ± 3.73
E. helioscopia	33.67 ± 3.78a	0	0	0
E. peplus	34.67 ± 8.02a	0	0	0
		Dasineura ex. Euphorbia virgata - Alland (Austria)		
GROUP V b/				
E. esula-virgata Austria (Control)	15.50 ± 4.24a	6.50 ± 2.12a	43.05 ± 1.96a	5.20 ± 3.10a
E. esula-virgata Montana biotype	14.67 ± 6.11a	4.00 ± 5.29a	29.17 ± 31.46a	4.81 ± 3.68a
E. esula-virgata Wyoming biotype	9.00 ± 1.41a	5.50 ± 4.95a	57.50 ± 45.96a	3.75 ± 2.10a
		Dasineura ex. Euphorbia virgata - Debrecen (Hungary e/)		
GROUP VI b/				
E. esula-virgata Nebraska biotype	11.00 ± 4.58a	0	0	0
E. esula-virgata Montana biotype	20.00 ± 11.36b	3.67 ± 3.51	27.38 ± 28.64	4.05 ± 3.15

a/ Means followed by the same letter within a column are not significantly different ($P < 0.05$; Student-Newman-Keuls (a posteriori) test); $\bar{X} \pm SD$ (n=3)

b/ Plants tested in 1984

c/ Plants tested in 1985

d/ % infestation = GP:FBL x 100 (GP = gall present; FBL = Flower buds left for gall development).

e/ Plant control not available

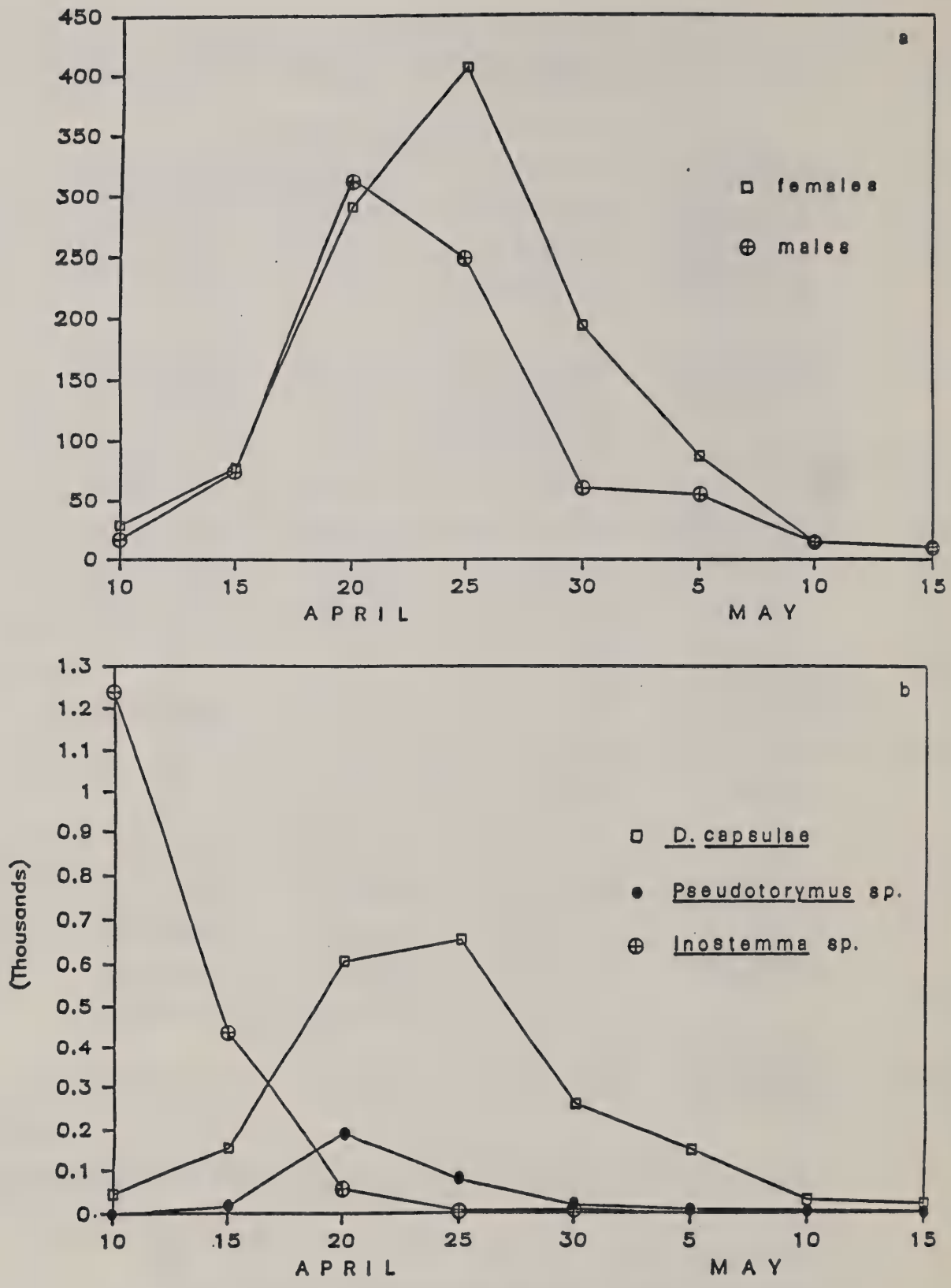


Fig. 1 - (a) Emergence curves of D. capsulae male and females. 1985 laboratory rearing.
(b) Emergence curves of D. capsulae and its parasites

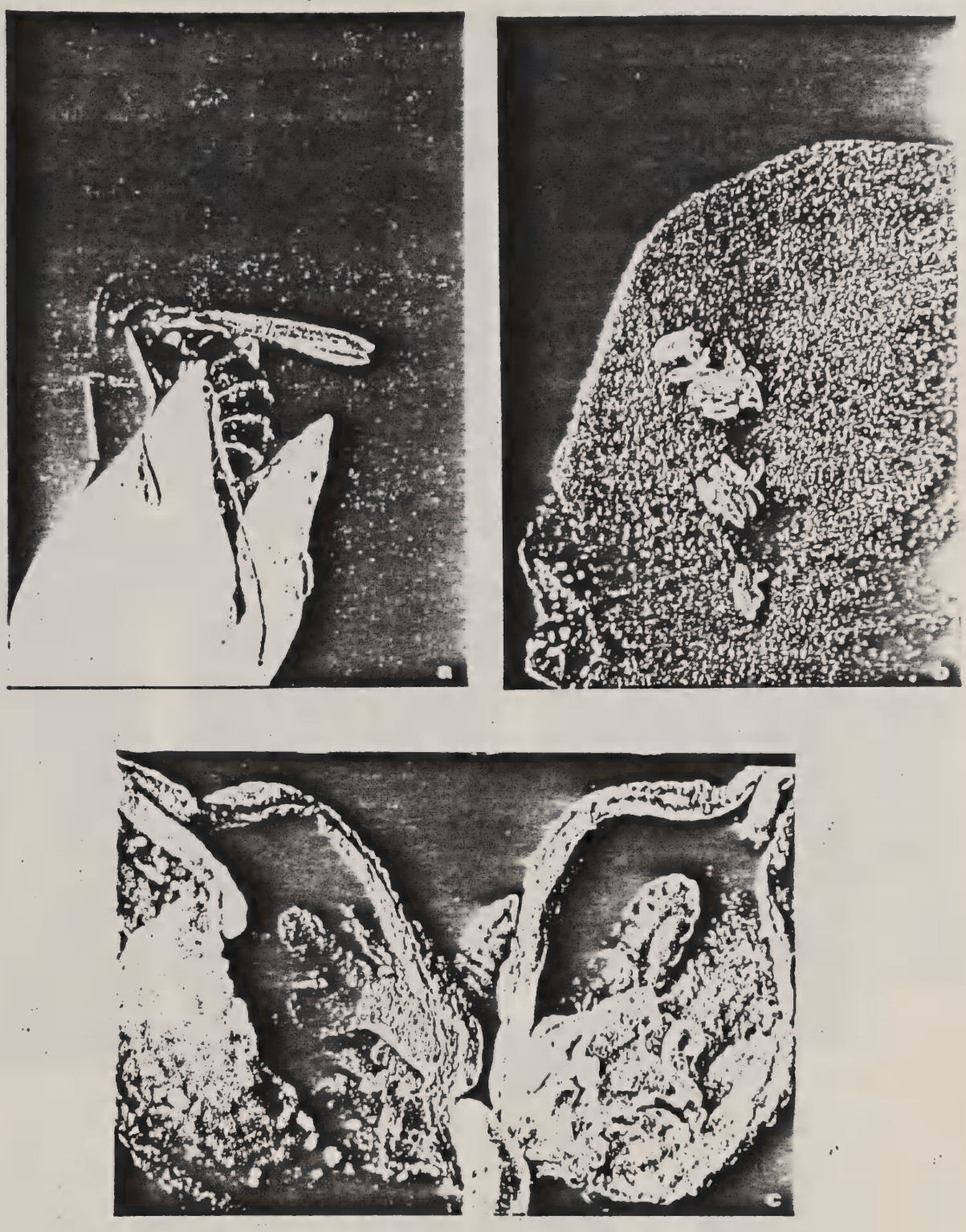


Fig. 2 - D. capsulae adult (a), eggs (b) and mature larvae within a gall.

Fig. 3 - Flower buds of the test-plants on which oviposition and larval development by D. capsulae occurred: (a) Euphorbia esula (Control), L = 4 mm; (b) E. esula - virgata Montana biotype, L = 5 mm; (c) E. esula virgata Wyoming biotype, L = 6 mm; (d) E. terracina, L = 4 mm; (e) E. lucida, L = 7 mm; (f) E. dendroides L = 5 mm.

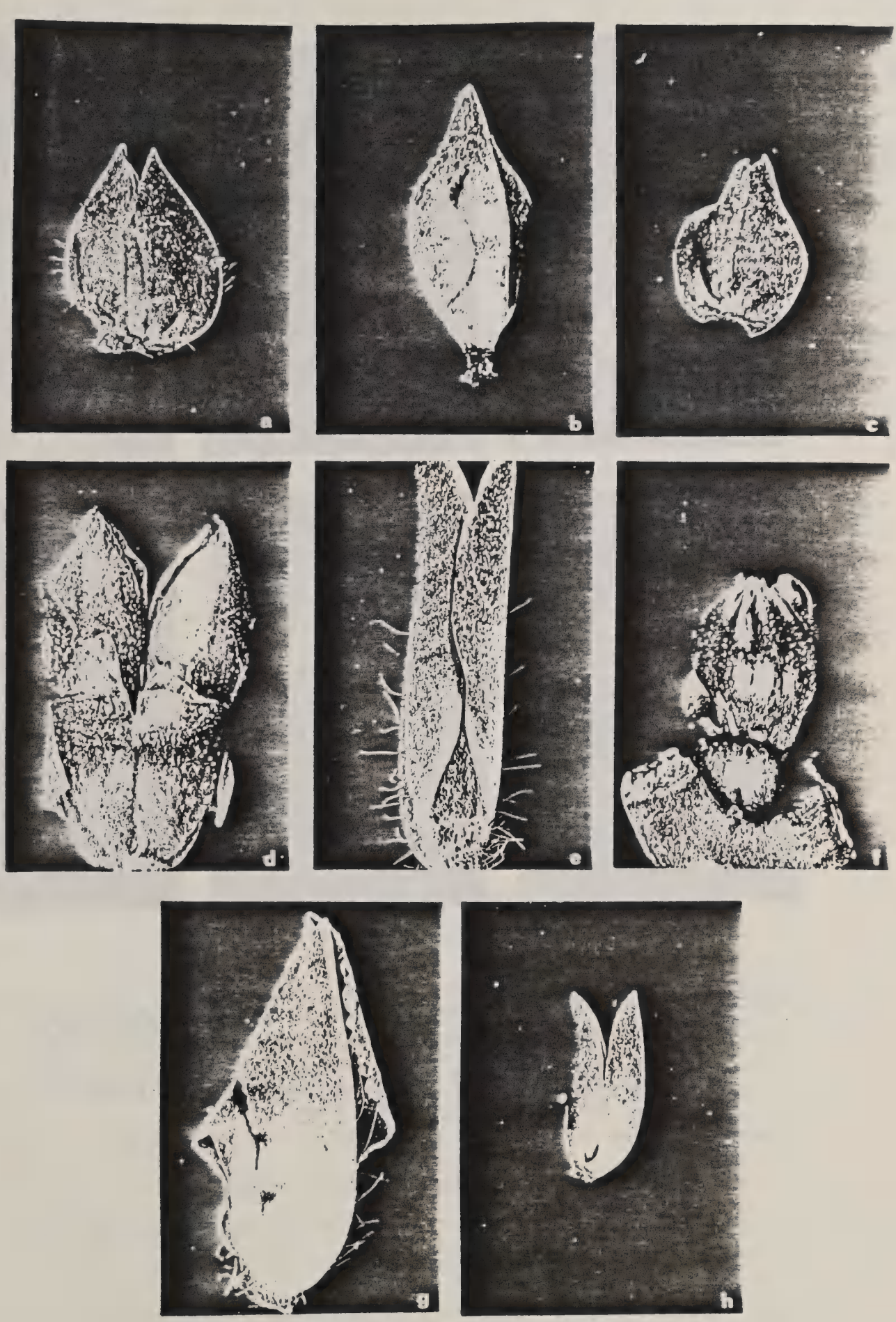


Fig. 4 - Flower buds of the test-plants on which only oviposition by D. capsulae occurred: (a) Euphorbia esula (Control), L = 4 mm; (b) E. esula - virgata Nebraska biotype, L = 5 mm; (c) E. amygdaloides, L = 3.5 mm; (d) E. milii, L = 4 mm; (e) E. marginata, L = 13 mm; (f) E. pulcherrima, L = 4 mm; (g) E. helioscopia, L = 7.5 mm; (h) E. peplus, L = 3 mm.

Fig. 5 - Flower buds of the test-plants of the genus Euphorbia not attacked by D. capsulae: (a) Euphorbia esula (control), L = 4 mm; (b) E. characias, L = 4 mm; (c) E. lathyris, L = 13 mm; (d) E. supina, L = 3 mm; (f) E. maculata, L = 4 mm; (g) E. serphyllifolia, L = 3,5 mm.

Chamaesphecia sp.

P. Pecora, M. Stazi, and M. Cristofaro

INTRODUCTION

In 1960's two clearwing moths, Chamaesphecia empiformis Esper, associated with Euphorbia cyparissias L., and C. tenthredimiformis Den. &. Shiff., associated with E. esula were selected as candidates for the biological control of leafy spurge (E. esula "complex") in North America. Host-specificity studies, conducted on both species, with populations from Eastern Austria, showed a restricted host-range, within the genus Euphorbia. C. empiformis reached the last instar only on the control (E. cyparissias) and on E. polycroma A. Kerner; while C. tenthredimiformis completed the larval development only on E. esula L. (Schroeder, unpublished data).

In 1970's both species were repeatedly released against leafy spurge in North America, but neither became established, probably because they were too host-specific to develop on the American biotypes of leafy spurge.

In 1982 a population of Chamaesphecia sp. was found on E. esula s.l. in Hungary by A. Rizza and P. Pecora. Neonate larvae of this insect were tested with some North American biotypes of leafy spurge, but complete larval development occurred only on the control plant of Hungarian origin. These results confirmed the high degree of specialization of the Chamaesphecia spp. associated with Euphorbia spp.

Taxonomic investigations showed that leafy spurge which occurs in North America is not a single species, but a complex of forms, species and hybrids. Apparently, the most common weed type seems to be E. x pseudovirgata Schur. (Soo), which is a hybrid between E. esula and E. virgata Wald. & Kit.

Since 1978, when the leafy spurge project was assigned to the USDA Rome laboratory, surveys have been made in Eastern Europe, the native home of leafy spurge, in an attempt to discover a population of Chamaesphecia sp. on E. x pseudovirgata. In 1984, a population of this clearwing moth was found on E. x pseudovirgata in Romania by P. Pecora and M. Cristofaro. Preliminary results of bionomic and host specificity studies made in 1985 are reported here.

Material and Methods

One hundred roots of E. x pseudovirgata, infested with various instars larvae of Chamaesphecia sp. were collected in Romania, in two localities near the Danube delta, at the end of October 1984. The infested roots were brought to the Rome laboratory and placed in 22 cm diameter terracotta pots. Thirty-two pots were prepared and each pot received 5-8 roots. The potted plants were kept outdoors in the laboratory garden until mid-April 1985, then they were enclosed in a large Saran cloth screen cage (2.00 x 2.00 x 2.00 m), and checked daily for adult emergence. To investigate on the life cycle of this clearwing moth, all the infested plants, from which adults emerged in the summer of 1985, were dissected at the end of January 1986, recording the number of individuals which were still in the larval stage.

In order to get biological data on this population of Chamaesphecia; i.e. preoviposition period, egg production/female, % of egg hatch, pre-eclosion period, adult longevity, newly emerged adults were caged on potted plants of E. virgata "group", in transparent plastic cylinders (D = 20 cm; H = 60 cm) with cloth covers. Eleven cages were set up with 1-2 ^{oo} and 1-3 oo/cage, as shown in table 1. These cages were kept out of doors in a shaded area, where the temperature ranged from 11^{o}C to 35^{o}C and RH from 20% to 90%.

When the eggs were found, they were removed from the oviposition site and transferred into 128-ml plastic cups, with a layer (2 cm thick) of moistened plaster of Paris on the bottom and capped by a plastic lid.

To determine if neonate larvae of Chamaesphecia could develop on some North American biotypes of leafy spurge, a larval survival test was set up in the laboratory garden. Test plants of E. virgata "group" from Nebraska (n=22), Montana (n=35), Wyoming (n=6), Oregon (n=6) and control plants from Romania (n=22), collected in the same localities where the larvae of the clearwing moth were found, were transplanted in a cement basin (L= 6m; W = 2m; H = 1m). Three neonate larvae or three mature eggs were placed on each plant, the first ones being infested on July 10, and the last ones on August 17, 1985. On October 8, 1985, several test and control plants were dissected and the number of larvae found were recorded.

Results

The mature larva of Chamaesphecia moved from the root to the lower part of the stem, where they made an exit hole, and covered with frass, then pupated. When the adult emerged, it split the chrysalid skin and came out through the exit hole prepared by the mature larva. During emergence, the chrysalid skin was dragged outside of the stem for about 3/4 of its length and held in place by anal hooks while the moth freed itself.

Thirty-two adult Chamaesphecia (13^{oo} 19 oo) emerged from June 16 to August 1, 1985. The preoviposition period ranged from 1 to 3 days, mean number of eggs/female was 29.33 ± 27.85 (n=9); (range=3-68), and the oviposition period lasted 1 to 5 days. The pre-eclosion period was 11-16 days; on a sample of 357 eggs 61.7% were fertile. Females lived 5.15 ± 2.12 days (n=13); (range=2-9); while male longevity was x 7.05 ± 1.96 days (n=19); (range=4-11).

Plants dissected (n=52) at the end of January 1986, disclosed 33 Chamaesphecia larvae, indicating that some individuals of this population have probably a biennial life cycle.

Results of larval survival tests, conducted with some North American biotypes of leafy spurge, showed the ability of larvae of this clearwing moth to develop on these plants, because the 30% of the test and control plants dissected were found to be infested with various instars of Chamaesphecia larvae (Table 1). This information allowed us to conclude that we have found the species or biotype of Chamaesphecia which will develop on the American varieties of leafy spurge.

To get a correct taxonomic determination, specimens of this insect were sent to the Sesiid specialist, Dr. C. Naumann (West Germany). If this clearwing moth is determined as empiformis or tenthrediniformis, the colony that we have on hand, will be shipped to the USDA/BCWL at Albany, California for field release. If it is identified as a third species it will be kept at the Rome laboratory to conduct additional host specificity tests to verify its safety. If the results of these trials are favorable a petition for introduction and release will be prepared.

Table 1. Results of dissections of North American biotypes of leafy spurge plants infested with Chamaesphecia larvae.

Test-plants	Source	# of plants dissected	# of plants infested	Total # of larvae found
Euphorbia pseudovirgata	Romania (control)	10	3	3
E. esula-virgata	Nebraska	10	3	3
"	Montana	20	6	6
"	Wyoming	6	2	2
"	Oregon	6	2	2

Euphorbia esula virgata "complex" (Leafy spurge)

P. Pecora, M. Cristofaro, M. Stazi

Collection trips:

a) In Italy - Five collection trips were made to S. Rossore (Pisa) by M. Cristoforo and M. Stazi from early May to the end of June, to collect the gall midge Bayeria capitigena (Bremi), the longhorned beetle Oberea erythrocephala and the flea beetle Aphthona flava Guill. on E. esula L.for release as biocontrol agents against leafy spurge in North America.

Three shipments of tip galls, containing various instars of B. capitigena were shipped to Albany, CA. on May 13 (150 galls), June 3 (200 galls), and July 7 (230 galls). Two workers spent four days making these collections.

Adults of the flea beetle A. flava were sent to Albany, CA on June 17 (n=230) and July 10 (n=1200). Two workers needed three days to collect these insects from E. esula.

One hundred-sixteen adults of O. erythocephala were shipped to Albany CA on June 17. One full day was spent by two workers to collect these insects.

b) Other countries

Three collection trips were made by P. Pecora and M. Cristofaro to Eastern Europe to provide the Albany laboratory with cold-hardy strains of some of the biological control agents associated with leafy spurge. The first trip lasted from June 19 to 27. Starting at the collection area, near Debrecen along the Romanian border, from June 20 to 23, a total of 15 Euphorbia virgata sites were visited. The population of O. erythrocephala was low, the number of insects collected ranging between 3 - 8 individuals per site. Thirty-seven Oberea erythocephala adults were found at 5 sites.

Bayeria capitigena

Five hundred Bayeria capitigena tip galls were collected on E. virgata at 6 of the 15 sites visited. The B. capitigena infestation was usually low, allowing a collection of about 25 galls from 500-1000 leafy spurge plants present at each site. However, in one site, South of Debrecen, we were able to collect 300 galls from ca. 3000 E. virgata plants. In order to have an indication on the number of larvae present in a gall, twenty-five mature galls from each site were dissected in the field. A range of 2-6 larvae per gall were found, a number considerably lower than the number of larvae (range= 5-30 larvae/gall) found on E. esula at S. Rossore (Italy).

A total of 45 larvae of Hyles euphorbiae L., of various instars were collected on E. virgata. This insect was present at only three sites, and was more common on E. cyparissiae L.. For example, in one site South-West of Debrecen, ca. 200 larvae were counted, but not collected, on this host-plant. On June 24 all the collected material was mailed to the Rome Laboratory from the American Embassy in Budapest.

We flew to Vienna in the afternoon of the 24th, where we rented a car, to go to the collection area in Austria. Two days were spent near St. Polten to collect 150 adults of the flea beetle Aphthona cyparissiae (Koch) on E. cyparissiass. In addition, 500 Dasineura capsulae Kieffer flower bud galls were collected on E. virgata Wald. & Kit. near Alland (west of Vienna). This material was sent to the Rome laboratory from the American Embassy in Vienna. We flew back to Rome on June 27.

A second trip to Hungary was necessary because the first shipment of Bayeria galls, collected in Hungary and sent to USDA-BCWL in Albany, California, on June 26, was lost at the New York airport. On July 11, we flew to Vienna and then left for Hungary by rental car. We spent two days at the

sites of the previous trip, collecting 400 galls on E. virgata and 300 on E. cyparissiass. The material was shipped to Rome through the American Embassy in Budapest. On June 14, we drove back to Vienna and spent another day collecting 140 adults of the flea beetle A. cyparissiasiae on E. cyparissiass. This material was sent to Rome from the American Embassy in Vienna to Rome where it was repacked and shipped to the USDA-BCWL, Albany, California.

A third trip was made to Romania and Austria to collect insects associated with leafy spurge and Canada thistle. On October 13 we flew from Rome to Bucharest. In the afternoon, we reached the collection area near Braïla, close to the Russian border.

Two days were spent collecting 100 infested roots of E. virgata with various instars of Chamaesphecia sp. Eighty roots were collected near Braila and the other 20 near Focsani. Of the 20 sites visited Chamasphecia larvae occurred only in two.

Also, 27 larvae of the noctuid moth Oxycesta geografica F. were collected on E. virgata. We had expected to collect more material of this insect, but the weather conditions (too cold) precluded a better collection.

On October 18 the collected material was mailed to Rome from the American Embassy in Bucharest.

Urophora cardui

On October 18 we flew to Vienna and drove to the collection area of Urophora cardui (L.), in a forested area near Vienna. In 3 days we collected 600 U. cardui galls on Cirsium arvense Scop. (Canada thistle) from 25 sites each of which had 1000-2000 (estimate) plants. Compared to the other years, when it was easy to collect 3000-4000 galls in two-three days the population of U. cardui in 1985 had decreased significantly.

The work in Austria and Hungary was greatly facilitated by the cooperation of Mr. James Freckman, the Agricultural Attache at the American Embassy in Vienna, Mr. F. Nemesc, Agricultural Assistant in Budapest, and Mr. A. Pavel, Agricultural Assistant in Bucharest.

Introduction

Yellow Starthistle Project

Stephen L. Clement

Research on the Eurasian phytophage associates of yellow starthistle (YST), Centaurea solstitialis L., has identified a promising complex of insects associated with the buds and flowerheads of YST (see ensuing reports). Two insects from this complex Urophora sirunaseva Hg. (Diptera: Tephritidae) and Bangasternus orientalis Cap. (Coleoptera: Curculionidae), were found to be host specific and subsequently released (1984-5) in western United States. Both insects came from Greece. Other members of the flowerhead complex are under study to determine their worth as biocontrol agents.

Surveys of YST were initiated in 1983 to augment those done earlier by C.I.B.C. and other USDA researchers. The aim of these surveys was to increase our knowledge of the insects attacking YST early in the growing season and parts of the plant other than flowerheads. A biological control agent drawn from such a pool of insects would supplement the damage caused by the two aforementioned flowerhead insects and other flowerhead insects that may be cleared for release in the U.S.

Three insects were selected for expanded studies in 1985 because the available data indicated they had the potential to become candidate biocontrol agents. These were two flowerhead insects, Chaetorellia hexachaeta (Diptera: Tephritidae) and Eustenopus hirtus (Waltl) and Apion weevils that feed as larvae in the roots and crowns of rosette stage plants. The research objectives for 1985 were, in large part, designed to learn more about these insects.

The planned research objectives for 1985 were:

1) To complete field and laboratory studies on the biology, host specificity and taxonomy of the Apion weevils associated with yellow starthistle in Italy and Greece.
2) To continue collaborative research with taxonomists, supplying reared material from known host plants, for the weevil and trypetid fly species complexes associated with rosettes and seed heads of yellow starthistle and related plants in Italy and Greece.
3) To initiate laboratory host specificity studies in Rome quarantine laboratory on Chaetorellia hexachaeta, a trypetid fly that attacks the flowerheads of yellow starthistle in Greece.
4) To initiate laboratory experiments to determine if the three most promising biological control agents of yellow starthistle (Urophora sirunaseva, Bangasternus orientalis, and Chaetorellia hexachaeta) compete for the same size and growth stages of buds and flowerheads of the host plant.
5) To conduct an open field trial in Greece using different varieties of yellow starthistle and three other plant species to gather data on the host preferences of Chaetorellia hexachaeta and Eustenopus hirtus.
6) To establish another field planting of different varieties of yellow starthistle in the garden at the Rome Laboratory to measure the preferences of insect natural enemies for these varieties.

The research under objective #4 was not done. Instead, the adult feeding behavior and host specificity of Eustenopus hirtus (Waltl) and Larinus nr. curtus Hochhut (Coleoptera: Curculionidae) was studied.

The research objectives are discussed in the following reports. A final report tries to identify the most promising candidate agents for expanded study in 1986 and beyond. This final report will also bring the reader up-to-date on the status of the YST project in Rome.

Apion weevil associates of *Centaurea solstitialis*

Mimmocchi and Clement

Research objectives for the 1985 research season were (1) to conduct female oviposition tests in the laboratory, (2) conduct additional larval survival and development tests in the laboratory, (3) to continue to study the biology of the main species on yellow starthistle (YST) at Castel del Monte (Puglia region), Italy, (4) to survey closely related thistle species and collect *Apion* weevils on these plants, and (5) to monitor Dr. Alonso-Zarazaga's progress to straighten-out the taxonomy of the *Apion* associates of yellow starthistle and closely related thistles in southern Europe and Turkey. Dr. Alonso-Zarazaga has funding from the Rome Laboratory to conduct taxonomic studies.

This report summarizes the progress we made under each objective. The reader can study the 1983 and 1984 annual reports of the Rome Laboratory for background information and the results of previous work.

METHODS AND RESULTS

Objective 1

It was necessary to go to the field and collect beetles for the laboratory oviposition test. This task, however, turned out to be a difficult one because the beetles were hard to find and collect during the April oviposition period in Puglia. We managed to collect five females, each of which was found clinging to the underside of a stage 3 rosette. Another four females were collected at Castel del Monte and another one about 12 km distant at a site supporting just a few YST rosettes but a goodly number of *Centaurea calcitrapa* plants. Dr. Alonso-Zarazaga identified these females and the ones that provided eggs and larvae for 1984 tests, as *Ceratapion basicorne*.

On April 12, these females were caged with small YST rosettes (one beetle per 500-cc cardboard container and rosette) and on April 14 the plants were examined and the beetles were kept in the cardboard containers without food or oviposition substrates for ca. 24 hours. Then, on April 16, three females were each individually caged with a young Carthamus tinctorius plant (all had less than 8 leaves) and two were individually caged with a stage 3 YST rosette. Cages were 1000 ml plastic beakers or clear plastic cylinders (width 20 cm; height 25 cm). After 7 days, on April 23, the five potted plants were dissected to record the number of eggs laid by each female, then the process was repeated for another 7 days. Lastly, between April 30 and May 7, each female was caged (plastic cylinders) with a young safflower plant (height 15 cm.) and a stage 2 YST rosette to record oviposition in a choice test. For this test the two test plants were grown together in a 22 cm clay pot. These tests, and others described in this report, were conducted in the quarantine greenhouse under temperatures of 18 $\pm$ 10°C, 60 $\pm$ 25% RH and ca. 14 h of natural light.

Table 1 is a summary of the results of the no-choice and choice oviposition tests. This table shows that three of the females readily laid eggs on young safflower plants under no-choice conditions; these eggs were viable and many of the larvae completed their development in the plants. On the other hand, these same three females did not lay eggs on safflower when given a choice between it and YST. Also, adults readily fed on safflower in the no-choice tests, but no feeding took place on the plant in the choice test.

The oviposition behavior on YST was the same in 1984 and 1985. Females made small slits in the midrib of leaves close to the crown. Then, eggs were inserted through these slits into small cavities, which were covered by a thin transparent layer of leaf epidermis.

In our view, oviposition by a weevil species on a critical test plant, even under no-choice laboratory conditions, indicates that a narrow host specificity is in doubt for the insect. We surmise, however, that C. basicorne would experience difficulty in ovipositing on a safflower plant beyond ca. the 10 leaf stage, with its thicker epidermis and more woody-like tissue.

Objective 2

Table 2 provides details and results of the larval survival test with potted test plants. Neonate larvae for this test came from eggs laid in the oviposition test (Objective 1). A camel hair brush was used to transfer neonate larvae into small cavities made with a straight pin in the middle of YST rosettes. The infestation site in Carthamus, Galactites and Zinnia plants was a small incision in the stem, which was made, respectively, ca. 2 cm, 1 cm, and 7 cm above the stem base of each plant species. For Carduus, Cnicus and Lactuca plants, larvae were placed in small cavities in the midrib of central leaves close to the root neck.

Besides YST, larval development took place in cultivated safflower, Carduus and Galactites (Table 3). At dissection larvae were feeding in the crowns of YST, Carduus and Galactities and in the central pith of safflower stems where they formed long tunnels. None of the infested plants showed any signs of stress when they were dissected. Larvae died as first instars in the other test plant species.

Objective 3

The biology of the main species (C. basicorne) at Castel del Monte was first studied in detail in 1984. This year the work focused on the life history of the insect in relation to the growth of its host plant and natural enemies of the weevils.

Life History Studies: All studies were conducted at Castel del Monte (called site 6 in the 1984 annual report). However, we were forced to restrict our sampling to a small portion of the 1984 study site (open 3.5 ha. field) because wheat was growing in most of it. Our sampling area (ca. 400 m^2) was divided into 3 equal sized strips and 10 $1m^2$ plots per strip were established on each sampling date (April 10, May 7 and 28) and all YST plants with 7 leaves were dug up from each plot (30 plots per date). New plots were established on each date; a distance of 5 m separated plots. Plants from each plot were placed in a numbered plastic bag. All plants were dissected in Rome to record the number of eggs, larvae, pupae and adults. The growth stages of YST were recorded to relate weevil activity with host plant phenology. Weevil larvae were preserved in 70% ETOH for head capsule measurements.

Eggs (n=33) were found on April 10 and as in 1984 they were inserted into the midribs of leaves and close to the crown. Ceratapion onopordi (Kirby, 1808) is another species that attacks YST but it inserts eggs into the root of the plant, about 1-3 cm below the root neck. In 1985, C. basicorne probably started to lay eggs in late-March and continued through April.

Fig. 1 shows the frequency of the three larval instars of C. basicorne on 3 sampling dates in 1985. This information is also shown for 1984. Some first instars were recovered on May 7, but most larvae (72.4%) were 3rd instars by that time. Mature larvae were generally found in the crowns.

Larval stem feeding was observed in plants that had not been fed upon by sheep. Pupae (n=14) were recovered May 28. Fig. 2 depicts the general life cycle of C. basicorne in relation to the phenology of YST at Castel del Monte. As observed in 1984, the first teneral adults appeared in the field when YST started to produce floral buds (late-May to early-June).

In 1983 and 1984 (Italy and Greece), we collected a few adults feeding on YST from mid-June to early-September; however, these were identified as Diplapion detritum (Mulsant and Rey, 1859) and D. stolidum (Germar, 1817) by Dr. Alonso-Zarazaga. Thus, we cannot provide any information about the status (adult aestivation, other feeding or breeding hosts, etc.) of C. basicorne after June.

Head capsule measurements were taken of the larvae and combined with those obtained in 1984 to produce Fig. 3.

Natural Mortality Factors: Twenty-seven eggs collected on April 10 yielded eight parasitoids all identified as Anaphes (Patasson) sp. (Hymenoptera: Eulophidae) by Dr. E.E. Grissell, Research Entomologist, USDA, Systematic Entomology Laboratory. On May 28 we recovered 3 larvae, 5 pupae and 1 adult of a larger hymenopteran parasitoid species. This pre-pupal or pupal parasitoid was identified as Trichomalus sp. (Hymenoptera: Pteromalidae) by Dr. Grissell. This information, although fragmentary, suggests that parasitoids might be a significant mortality factor for C. basicorne.

Objective-4

The only survey to record the presence-absence of C. basicorne on various thistle species was done in the study plot at Castel del Monte. Besides YST, the dominant plants in this plot were Onopordum acanthium and Carduus nutans. On May 7, 25 rosettes of each of these two species were collected and transferred to the Rome Laboratory where all of the C. nutans and 20 of the

O. acanthium plants were dissected. No curculionid larvae were found in Carduus but 92 larvae and 6 pupae were found in the Onopordum plants. Weevils reared out from the five undissected Onopordum plants and a few adult weevils collected on C. nutans rosettes were sent to Dr. Alonso-Zarazaga. None of these weevils were identified as C. basicorne.

Objective 5

Dr. Alonso-Zarazaga made significant progress towards clarifying the taxonomy of the species associated with YST and other thistle plants. Table 1 is a "preliminary summary (i.e. information subject to change)" of the host plants of the weevils submitted by us and identified by Dr. Alonso-Zarazaga. Clearly, quite a few species use YST as a feeding and breeding host. However, the main species on YST, and the one that has been associated most often with this plant in France, Italy, Greece, and Turkey, is Ceratapion basicorne (Illiger, 1807). It should be mentioned that one adult of C. basicorne was collected by S. Clement on a flowerhead of cultivated safflower in Greece; however, there was no evidence that this insect had fed on the plant. Specimens of two other species, C. orientale (Gerstacker, 1854) and Diplapion detritum (Mulsant and Rey, 1859), were only reared from YST but because very few larvae or beetles were encountered it may be difficult to obtain sufficient numbers of insects for host specificity tests. Only C. diffusa is known to be another host for C. orientale (Alonso-Zarazaga, pers. comm). Besides YST, Apion (Diplapion) detritum Rey is recorded from Arctium minor and Silybum marianum (see 1983 annual report); however, we cannot vouch for the authenticity of these records.

Table 1. Number of eggs laid in oviposition tests by five females of Ceratapion basicorne collected on April 10, 1985 in Puglia region, Italy.

	No-Choice Tests						Choice Test	
	April 12 - 14	April 15	April 16 - 23		April 23-30		April 30 - May 7	
Female No.	YST[a/]	No Substrate Offered	YST	SF[b/]	YST	SF	YST	SF
1	8	0	-	15	-	14	22	0
2	5	0	-	13	-	7	19	0
3	4	0	-	9	-	5	18	0
4	5	0	29	-	25	-	33	0
5	10	0	33	-	16	-	21	0
Total	32	0	62	37	41	26	113	0

a/ YST = Centaurea solstitialis
b/ SF = Carthamus tinctorius var. Hartman, U.S.

Table 2. Results of larval survival test with Ceratapion basicorne, Rome, Italy, April 15 - May 17, 1985.

Test Plants and Source of Seeds	Growth Stage and Size of Test Plants	No. of Plants Tested	No. Plants with Surviving Larva at Dissection 1/	Larval Instars Recovered at Dissection
Centaurea solstitialis Rome, Italy	Rosette, stage 3	4	3 (21-23) 2/	3rd instar
C. solstitialis Yakima, WA., USA	Rosette, stage 3	4	3 (21)	3rd instar
C. solstitialis Walla Walla, WA., USA	Rosette, stage 3	4	4 (21)	3rd instar
Carthamus tinctorius var. Hartman, USA	Height 10-13 cm 6-8 leaves	10	7 (21)	3rd instar
C. dentatus Thermi, Salonika, Greece	Rosette, spread 12-14 cm	5	0 (21)	-
Carduus pycnocephalus Trieste(Bot.Garden),Italy	Rosette, spread 26-30 cm	9	1 (21)	3rd instar
Galactites tomentosa Rome lab garden,Italy	Pre-bolt stage	5	1 (21)	3rd instar
Cnicus benedictus Trieste(Bot.Garden),Italy	Rosette, spread 40-50 cm	3	0 (21)	-
Lactuca sativa var. Bibb.(Limestone),USA	8 - 12 leaves	5	0 (21)	-
Zinnia elegans Trieste(Bot.Garden),Italy	Height 15-18 cm	5	0 (21)	-

1/ Each plant received one newly hatched larva.
2/ Figures in parentheses indicate days, post larval infestation, when plants were dissected.

Table 3. Host plants of Ceratapion and Diplapion weevils identified by Dr. Miguel A. Alonso-Zarazaga, 1986.

Weevil Species	Host Plants									
	Centaurea solstitialis	Centaurea stoebe	Centaurea diffusa	Centaurea nicaeensis	Carthamus lanatus	Carthamus tinctorius	Carduus nutans/thoermeri	Carduus pycnocephalus tenuiflorus	Silybum marianum	Onopordum acanthium
Ceratapion basicorne	L[1], A[2]					?[3], A				
C. scalptum					?, A	?, A			L, ?	
C. penetrans			L, A				?, A			
C. onopordi	L, A	L, ?				?, A	L, A	L, ?	?, A	
C. orientale	L, A									
C. robusticorne				L, A						
C. carduorum	?, A						L, A	L, A		L, A
C. tumidum	?, A		?, A				L, A	L, A		
Diplapion detritum	L, A									
D. stolidum	?, A									

1/ L = larval breeding host

2/ A = adult feeding host, or adult was resting on plant when collected.

3/ ? = no information available.

-1984-

-1985-

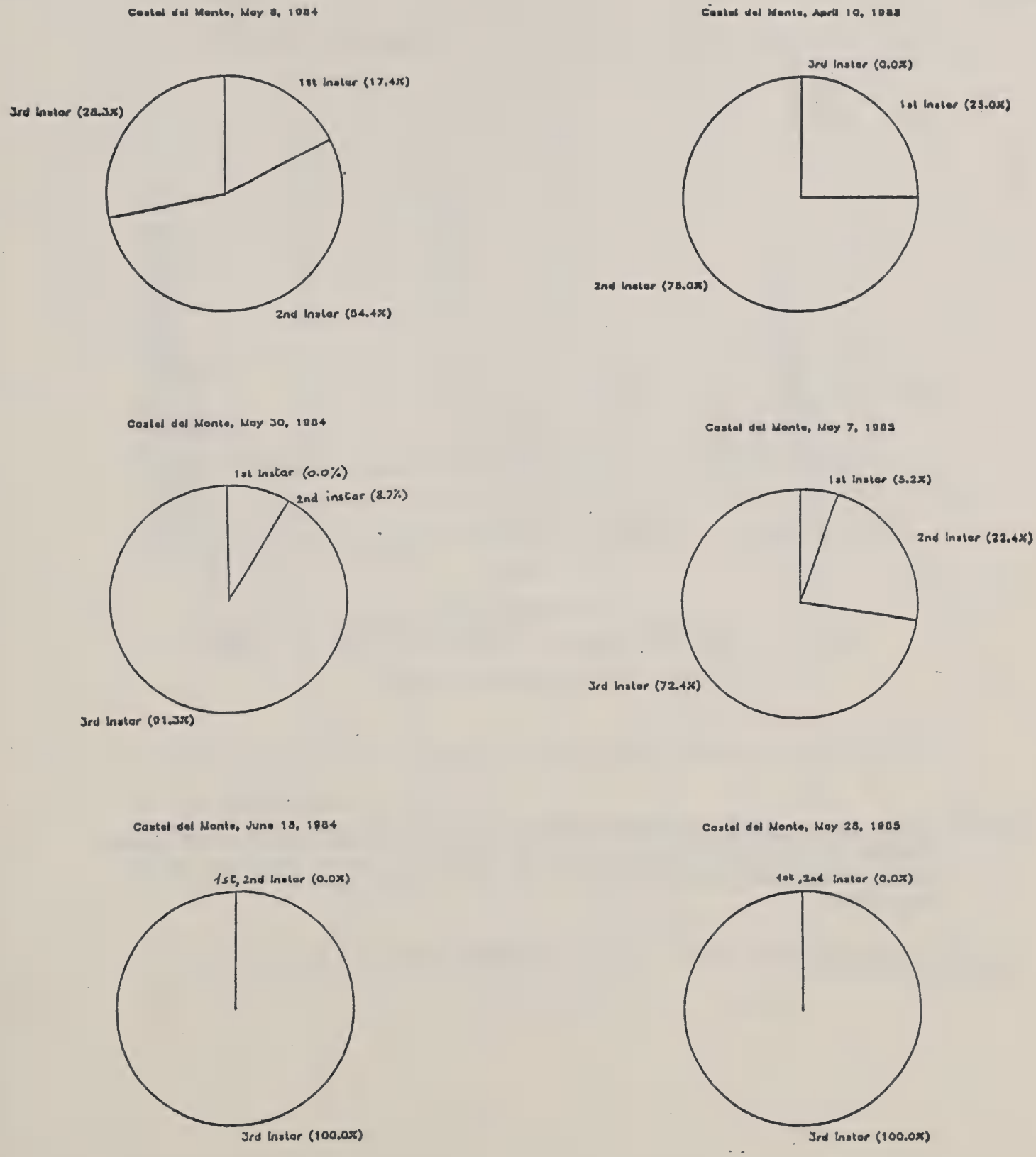


Fig.1: Pie charts showing the frequency of larval instars of Ceratapion basicorne on 3 sampling dates at Castel del Monte (Puglia), Italy, 1984 and 1985.

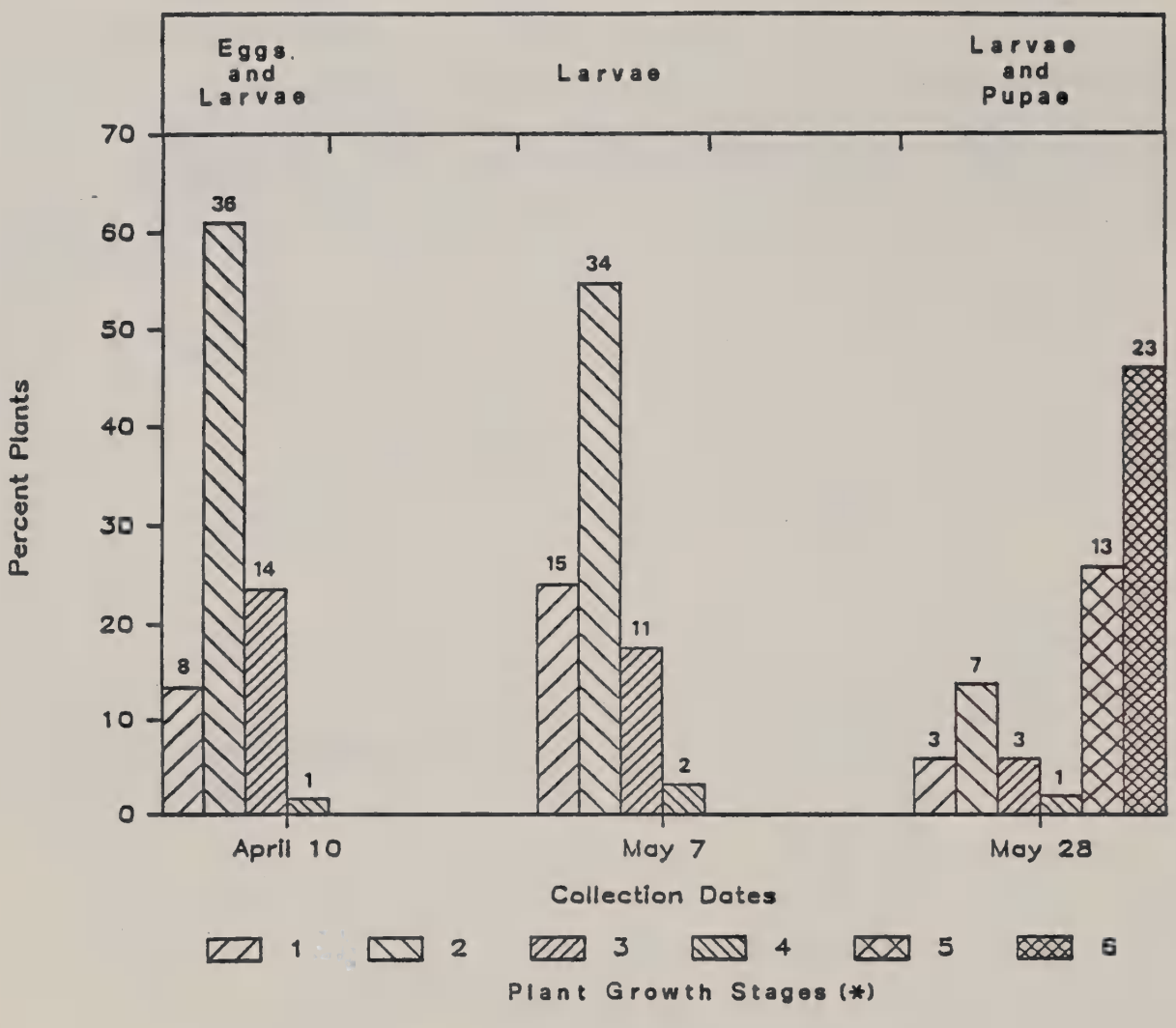


Fig.2: Ceratapion basicorne life history and frequency distribution of growth stages of Centaurea solstitialis at Castel del Monte (Puglia), Italy, 1985. Number of plants per growth stage is shown at top of each histogram.

(*) Plant growth stages according to the scheme described in 1984 annual report (pp. 37-38).

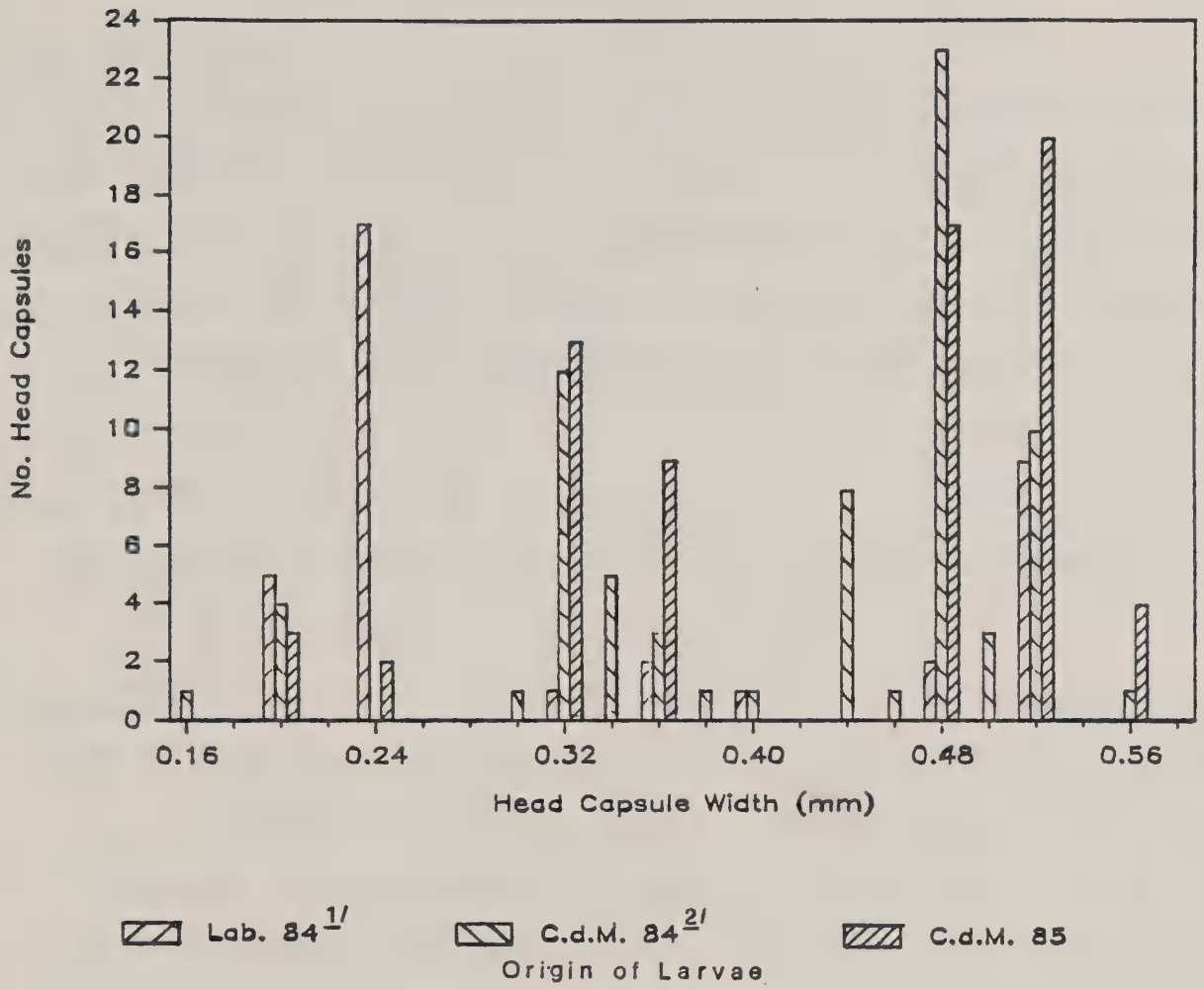


Fig.3: Frequency of Ceratapion basicorne larval head capsule widths, 1984-85.

1/ Laboratory reared.
2/ Field collected at Castel del Monte, Puglia, Italy.

Weed Garden-Plot Experiments

Clement, Mimmocchi, Sobhian and Cristofaro

Weed garden-plots were set up in 1983, 1984 and 1985 on the grounds of the Rome Laboratory and in 1985 on the Agricultural Research Farm, University of Thessaloniki, Greece . Research objectives for this rather large study were: (1) to measure diversity, abundance, and pattern of attack of the natural enemies of Centaurea solstitialis L. (YST) in central Italy and northern Greece; (2) determine the suitability of different strains of YST, cultivated thistle crop species (safflower and artichoke) and other closely related thistles (Cirsium spp.) as feeding, oviposition and breeding hosts for natural populations of the most promising candidate biocontrol agents of YST; (3) generate baseline data on the life history and natural enemies of certain herbivores; (4) examine variance in herbivore loads among strains of YST grown under uniform plot conditions; (5) delineate, if possible, competitive interactions among the phytophages comprising the flowerhead and seedhead guild; (6) observe and quantify the level of damage caused by individual species; and (7) take note of phenotypic plasticity in the various YST strains in the garden-plots.

During the course of our work we also discovered that "trap" plots weeds can be used to build up populations of some insect species. Armed with this knowledge overseas workers might want to use such plots to obtain sizeable numbers of insects for use in laboratory host specificity tests.

In this report we present much of the data generated by the garden-plots. A complete interpretation of the data will be reported in scientific journal articles.

Methods

Rome Garden-plots

The first Rome garden-plot was established on March 25, 1983 when 24 greenhouse-grown rosettes, representing 6 strains of *C. solstitialis* (source of seed was Brindisi, Italy; southern Spain; Walla Walla and Yakima, Washington; Concord and Tehama County, California), were transplanted into a plot 12 x 12 m. The six strains (treatments) were arranged in a 6 x 6 Latin square with 1 m between plants. The plot was surrounded by several hundred plants of the local strain of *C. solstitialis*.

The 1984 and 1985 Rome garden-plots occupied the same 12 x 12 m plot-space, about 50 m east of the 1983 plot-space. In both years, greenhouse-grown rosettes were transplanted into the plots between April 3-5. In 1984, seven strains of *C. solstitialis* (Rome, Italy; s. Spain; Walla Walla and Yakima, Washington; Lapwai, Idaho; Sacramento and Contra Costa County, California) and one cultivar of *Carthamus tinctorius* L. were arranged in an 8 x 8 Latin square with 1.5-2 m separating plants. The 1985 plot contained five strains of *C. solstitialis* (Rome; s. Spain; Thermi, Greece; Lapwai, Idaho; Sacramento, California) and one cultivar of *C. tinctorius* in each of 4 rows, using a randomized complete block design for a total of 24 plants. Row and plant spacing was 1.5 m. *Urophora* colonizing the 1984 and 1985 gardens most likely came from a nearby (50 m) planting of several hundred host plants of the local strain, as no other *C. solstitialis* was seen in the vicinity (0.5-1 km.) of the Laboratory.

Sampling was done by harvesting capitula in the flowerhead stage (see exception below for some branches on some plants in 1985 garden plot) on each plant at 3-7, 5-14 and 7-day intervals in 1983, 1984, and 1985, respectively. Each sample (=capitula from one plant each time) was placed in a labelled 500 cc. cardboard container fitted with a nylon organdy cover to retain emerging

insects and their parasitoids. These containers, kept in a laboratory room (temperature ranged from 15-32°C throughout the year) with a window without shade providing natural light, were checked 1-3 times per week to remove emerging insects. Parasitoids were placed in alcohol in labelled vials and the phytophages were pinned and labelled.

We discovered, in 1983, that predaceous spider populations are fairly high on YST, resting on buds and flowerheads to capture prey. Therefore, all spiders were removed from the flowerheads in 1984 and 1985 before the heads were placed in cups to rear out the phytophagous insects and their parasitoids.

The number of YST capitula dissected in 1984 and 1985 was, respectively, 26,067 and 10,678. Small capitula ($\leq$5 mm dia. at widest point) were not dissected in 1984 and 1985 because these heads in the 1983 dissections did not harbor seedhead insects. Dissections were done during the winter months to record the number of damaged capitula per sample. If a immature form was not in a seedhead, some insect feeding damage had to be evident before a head was classified as "damaged". We had no way of determing the number of eggs which failed to hatch or neonate larvae which died before feeding and leaving detectable damage. Thus, we cannot provide an indication of host acceptability; that is, the suitability of different YST strains and safflower as oviposition sites for the flower and seedhead guild that colonized the plots. We do provide, however, data on the suitability of a selected host plant for insect development (= host suitability).

All of the heads (7,309) from the 1983 garden-plot were dissected but the data do not accurately reflect levels of damage per YST strain because many small heads ($\leq$ 5 mm dia.) were categorized as "insect damaged" when in fact they were not. Methods were changed in 1984-85 to take into account the lessons learned in 1983.

In 1985 we tried to tabulate the number of YST capitula damaged by each species. To accomplish this it was necessary to identify the insect species present in a seedhead and associate damage with a particular species in the absence of an insect or its remains. This posed no problems in identifying the receptacle galls of U. jaculata, larvae of Lasioderma and damage of Chaetorellia and Acanthiophilus larvae and pupae, but we were unable to identify the tephritid larvae that destroyed some achenes before dying. Also, we could not accurately count the number of capitula with U. quadrifasciata galls in ovary walls. Moreover, a sizeable number of capitula were categorized as "damaged by unidentifiable larva, but most likely a tephritid".

All of the 1984 and 1985 YST samples from Rome were dissected by T. Mimmocchi and S. Clement. By the start of the 1984 season these workers were able to identify the common occupants of YST capitula, and the type of damage they cause inside YST flowerheads.

Three plants of each YST strain in the 1985 garden-plot were chosen with the aid of a random number generator. On June 4 three branches were chosen on each plant; these were labelled "low", "middle" and "high" because branches were selected in the lower, middle and upper portions of a plant. Bud and flower development was followed on each plant by counting the number of Bu-1, -2, -3 and -4 buds and F-1 and F-2 flowerheads (stages according to Maddox 1981) on each branch at 7-day intervals from June 4 to August 19. Capitula in the seed formation stage were collected on each sample date.

The ground around and between the test plants was periodically hoed or machine-tilled to remove weeds. Occasional rain was the only source of water for the plants.

Thermi Garden-plot

The Thermi, Greece garden-plot (12 x 12 m) contained three strains of C. solstitialis (Thermi; Lapwai, Idaho; Sacramento, California), Cirsium creticum (Lam.) D'Urv., Cynara scolymus L., and Carthamus tinctorius in each of 6 rows, using a randomized complete block design for a total of 36 plants. Rosettes of C. solstitialis were transplanted into the garden on March 11, 1985. Row and plant spacing was ca. 2 m. There were a few wild plants of yellow starthistle near the plot (within 5 m) and a moderate sized patch (ca. 50 plants) about 10 m away.

Dr. Sobhian's portion of this Annual Report provides a more detailed account of the procedures employed to establish this plot, the methods used to follow bud and flower development on the three YST strains, and other methods for collecting data on the host plant specificity and bionomics of Chaetorellia hexachaeta australis Hering and Eustenopus hirtus (Waltl.).

RESULTS

Rome Garden-plots

Species Diversity and Relative Abundance: Tables 1, 2 and 3 depict the taxonomic composition of the insects that emerged from each YST strain in the 1983, 1984 and 1985 garden-plots. The insects that emerged from cultivated safflower in 1984-85 are also shown.

More Acanthiophilus helianthi (Rossi) (Diptera: Tephritidae), a polyphagous fly, were reared out than any other insect in 1983 (Table 1) but our findings from that year must be interpreted with caution because a goodly number of insects were destroyed by spiders in the laboratory rearing containers.

The spiders were removed from 1984 and 1985 samples before they were placed in cups. Thus, the results in Tables 2 and 3 accurately reflect the abundance of the insect species that emerged in the laboratory during the summer and fall of 1984 and 1985. Overwintering insects were not allowed to emerge because the capitula had to be dissected during the winter months to leave time for other work in the spring and early summer months. Even if the overwintering insects had been allowed to emerge we do not think that species diversity or the relative species abundance rankings for each YST strain as, depicted in Tables 2 and 3, would change significantly because a disproportionately large number of overwintering forms were Chaetorellia sp. nr. carthami Stackelberg. In contrast, all overwintering insects in safflower heads were allowed to emerge so the counts in Table 2 and 3 reflect the total number emerging insects from C. tinctorius. The dominant species in 1984 and 1985 was Chaetorellia sp. nr. carthami (Tables 2-3), a tephritid fly that has already been disqualified as a candidate biocontrol agent (see Sobhian and Zwölfer 1985).

Table 4 was assembled with the data on Urophora jaculata Rondani and U. quadrifasciata (Meigen) in Tables 1-3. Just 9 flies of U. jaculata emerged but they all came from Italian plants. Flowerhead receptacle galls of this fly were only found in the dissected capitula from Italian plants. While YST is not a recorded host for U. quadrifasciata in North America where the fly was imported from Europe and is now established on two knapweed pests, Table 4 shows that Palearctic and Nearctic populations of YST are suitable hosts for a Rome population. According to Dr. I. White (Commonwealth Institute of Entomology, London), European populations of U. quadrifasciata on different Centaurea subgenera may "represent separate biological species that are not obviously distinct in morphology". What matters for biological control of YST is that U.S. plants are suitable hosts for at least one population of the fly presently known as U. quadrifasciata.

We are most grateful to the following persons for identifying the insects listed in Tables 1-3: Dr. I. White (CIE, London) for Urophora jaculata, Urophora quadrifasciata, Chaetorellia carthami Stackelberg (YST host race), Orellia n. sp. (nr. colon, agg. (Sp.B)), and Terellia n. sp. (nr. virens, agg. (sp.B)); Dr. R.H. Foote (Retired Research Entomologist, Systematic Entomology Laboratory, USDA) for Acanthiophilus helianthi (Rossi); Dr. R.W. Hodges (Research Entomologist, Systematic Entomology Laboratory, USDA) for Pyroderces argyrogrammos; Dr. R.W. Poole (Research Entomologist, Systematic Entomology Laboratory, USDA) for Eublemma parva; and Dr. R. Madge (Commonwealth Institute of Entomology, London) for Lasioderma sp. nr. haemorrhoidale. The identity of the one Metzneria moth (Table 2) was checked by a comparison with identified specimens (Det. R.W. Hodges) in the general insect collection at the USDA Biological Control of Weeds Laboratory in Rome. The identity of the Isocolus specimens in Table 2 has not been established by a specialist or comparison with identified material.

Herbivore Loads Among Strains of YST: The insect community that colonized the 1984 and 1985 garden-plots in Rome seems to be adapted to the YST strain which normally occurs in the vicinity of the Rome Laboratory. Figures 1-2 show that seasonal herbivore loads were greatest in the capitula of the Rome, Italy strain of YST. In general, the Spanish plants suffered the lowest percentage of damaged capitula. The U.S. plants were suitable hosts for the flowerhead and seedhead guild.

Phenotypic Plasticity in YST Strains: Bu-3 and -4 buds are the stages chosen for oviposition by the dominant taxonomic group (Tephritidae) that attacked YST. Figure 3 reflects the occurence of Bu-3 and -4 buds (expressed as the percent of the total counted for the season) on each strain over the period of bud development. Bud development was accelerated in the Idaho plants, whereas it was pretty much synchronized on plants of the other strains

Table 1 : Taxonomic composition of the insects that emerged from capitula of six strains of Centaurea solstitialis L. (YST), Rome, Italy 1983 .

Insect Taxa	Number of Emerging Insects — Host Plants: YST (5)* Rome, Italy	YST (6) Southern Spain	YST (6) Walla Walla, Washington	YST (6) Yakima, Washington	YST (6) Tehama, California	YST(4) Concord, California	TOTAL
Diptera: Tephritidae							
Acanthiophilus helianthi (Rossi)	6	10	16	7	10	0	49
Urophora quadrifasciata (Meigen)	7	0	7	2	0	0	16
Terellia n.sp.	4	1	2	0	1	0	8
Chaetorellia carthami Stackelberg (YST host race)	1	0	2	2	0	0	5
Urophora jaculata Rondani	1	0	0	0	0	0	1
Lepidoptera: Momphidae							
Pyroderces argyrogrammos Zell.	1	0	0	1	0	0	2
TOTAL	20	11	27	12	11	0	81

* Number in parentheses is number of plants (= replicates) per strain .

Table 2 : Taxonomic composition of the insects that emerged from capitula of seven strains of *Centaurea solstitialis* L. (YST) and one cultivar of *Carthamus tinctorius* L., Rome, Italy 1984 .

Insect Taxa	Number of Emerging Insects — Host Plants: YST Rome, Italy	YST Granadella, Spain	YST Lapwai, Idaho	YST Sacramento, California	YST Contra Costa, California	YST Walla Walla, Washington	YST Yakima, Washington	*Carthamus tinctorius*	TOTAL
Diptera: Tephritidae									
Chaetorellia carthami Stackelberg (YST host race)	41	11	37	118	40	138	94	0	479
Acanthiophilus helianthi (Rossi)	107	1	24	24	49	46	20	46	317
Urophora quadrifasciata (Meigen)	2	0	2	2	2	1	0	0	9
Urophora jaculata Rondani Rondani	3	0	0	0	0	0	0	0	3
Terellia n.sp.	1	0	0	0	0	1	0	0	2
Orellia n.sp.	0	0	0	0	0	0	0	13	13
Unknown Tephritidae	0	0	0	0	1	0	0	0	1
Coleoptera: Anobiidae									
Lasioderma sp.nr. *haemorrhoidale* (Illiger)	0	1	3	3	6	1	2	0	16
Lepidoptera: Momphidae									
Pyroderces argyrogrammos Zell.	0	0	0	0	1	0	0	0	1
: Gelechiidae									
Metzneria sp.	1	1	0	0	0	0	0	0	2
Unknown Lepidoptera	0	0	0	0	0	1	0	0	1
Hymenoptera: Cynipidae									
Isocolus sp.	3	0	0	0	0	0	0	0	3
TOTAL	158	14	66	147	99	188	116	59	847

Table 3 : Taxonomic composition of the insects that emerged from capitula of five strains of Centaurea solstitialis L. (YST) and one cultivar of Carthamus tinctorius L., Rome, Italy 1985 .

	Number of Emerging Insects						
	Host Plants						
Insect Taxa	YST Rome, Italy	YST Granadella, Spain	YST Thermi, Greece	YST Lapwai, Idaho	YST Sacramento, California	Carthamus tinctorius	TOTAL
Diptera: Tephritidae							
Chaetorellia carthami Stackelberg (YST host race)	130	64	28	50	49	0	321
Acanthiophilus helianthi (Rossi)	10	46	2	28	2	245	333
Urophora quadrifasciata (Meigen)	38	2	2	17	2	0	61
Urophora jaculata Rondani Rondani	6	0	0	0	0	0	6
Terellia n.sp.	0	0	0	1	0	0	1
Orellia n.sp.	0	0	0	0	0	113	113
Coleoptera: Anobiidae							
Lasioderma sp.nr. haemorroidale (Illiger)	1	15	0	2	3	24	45
Lepidoptera: Momphidae							
Pyroderces argyrogrammos Zell.	0	0	1	0	0	1	2
: Noctuidae							
Eublemma parva (Hubner)	0	0	1	1	0	0	2
TOTAL	185	127	34	99	56	383	884

Table 4. Number of adults of Urophora quadrifasciata (Meigen) and Urophora jaculata Rondani (Diptera: Tephritidae) that emerged from capitula of plants of different strains of Centaurea solstitialis L. (Asteraceae) grown together in experimental garden plots, Rome, Italy 1983-1985.

Source of Seed for the strains of Centaurea solstitialis	Number of Flies per Year					
	Urophora quadrifasciata			Urophora jaculata		
	1983	1984	1985	1983	1984	1985
Europe[a/]						
Central and Southern Italy	7	2	38	1	3	6
Southern Spain	0	0	2	0	0	0
Northern Greece	[c/]	-	2	-	-	0
United States[b/]						
Washington State	9	1	-	0	0	-
Idaho State	-	2	17	-	0	0
California State	0	4	2	0	0	0

a/ Seeds of European strains came from plants growing in Brindisi (1983) and Rome (1984 and 1985), Italy; southern Spain (1983-85),; and Thermi (1985), Greece.

b/ Seed of U.S. strains came from plants growing in Walla Walla and Yakima (1983-1984), Washington; Lapwai (1984-85), Idaho; Concord and Tehama County (1983), Sacramento (1984-85), and Contra Costa County (1984), California.

c/ Strain not grown

of YST. These data suggest that phenotypic plasticity (with respect to bud development) among the YST strains was insignificant and thus was not associated with the observed differences (Fig. 3) in herbivore loads. The tentative conclusion is that there is genetic heterogeneity in YST and this influences the degree to which a strain is a suitable larval host for the insect community in Rome. Further scrutiny of the data will confirm this suspicion or lead to an alternative conclusion(s).

Another way that we tried to detect phenotypic plasticity was by comparing the width and height of mature plants and numbers of flowerheads produced in a season by plants of each YST strain. Table 5 gives these measurements and counts for the seven and five strains in the 1984 and 1985 garden-plots, respectively. Although the 1984 plot was arranged as an 8 x 8 Latin square with Carthamus tinctorius as one of the treatments, the statistical analysis (ANOVA) was done according to a RCB design, as we were only interested in the YST strains. Table 5 shows that there were no block differences ($P > 0.05$) but there were real treatment (among strain) differences in plant height and number of flowerheads in 1984 ($P < 0.01$) and plant height in 1985 ($P < 0.05$). An interesting aspect about this data is that the most "suitable" hosts, the Italian plants, were some of the smallest plants in terms of width and height and produced, on the average, less flowerheads than plants of some of the other larger strains.

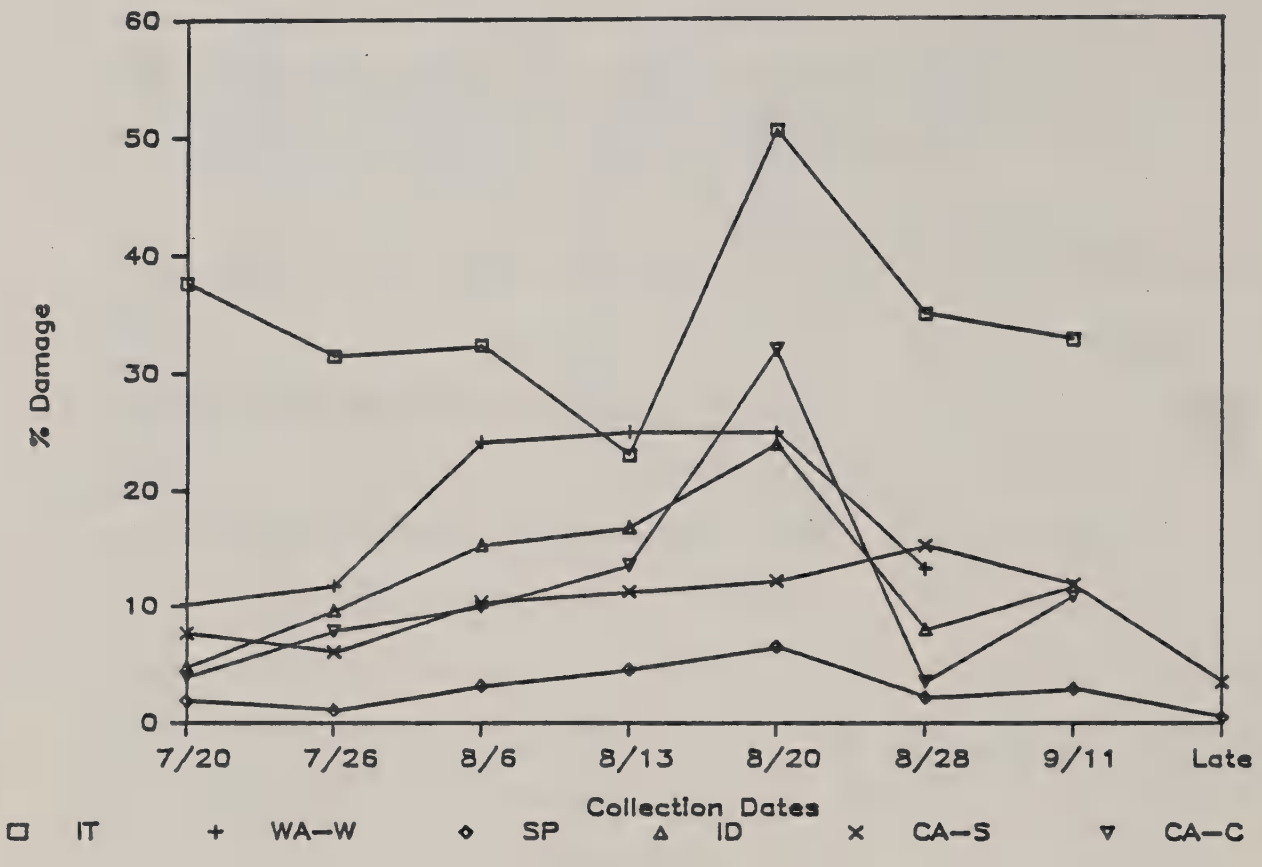


Fig. 1 : The percentage of insect damaged capitula from plants of six strains of Centaurea solstitialis L., July 20 through late season (September 24 to October 15), weed garden-plot study, Rome, Italy 1984 .

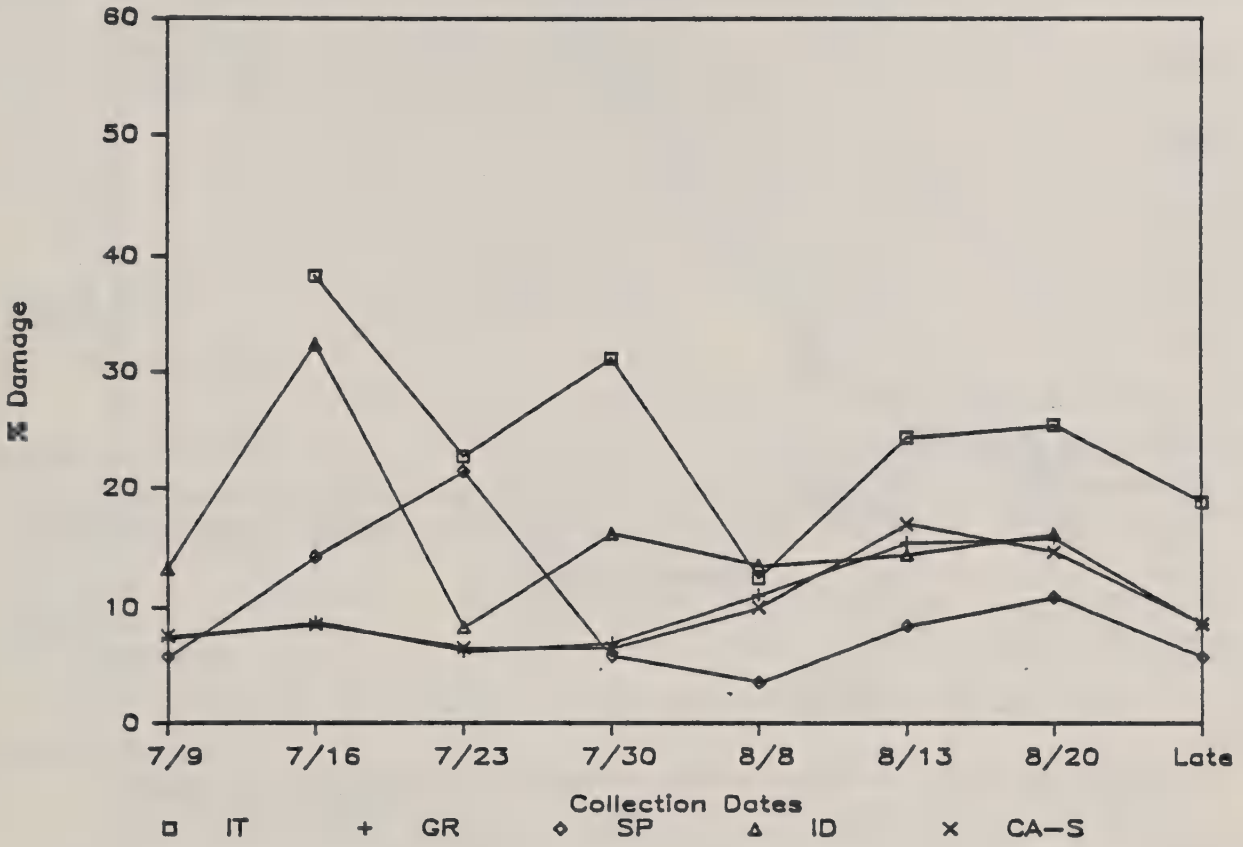
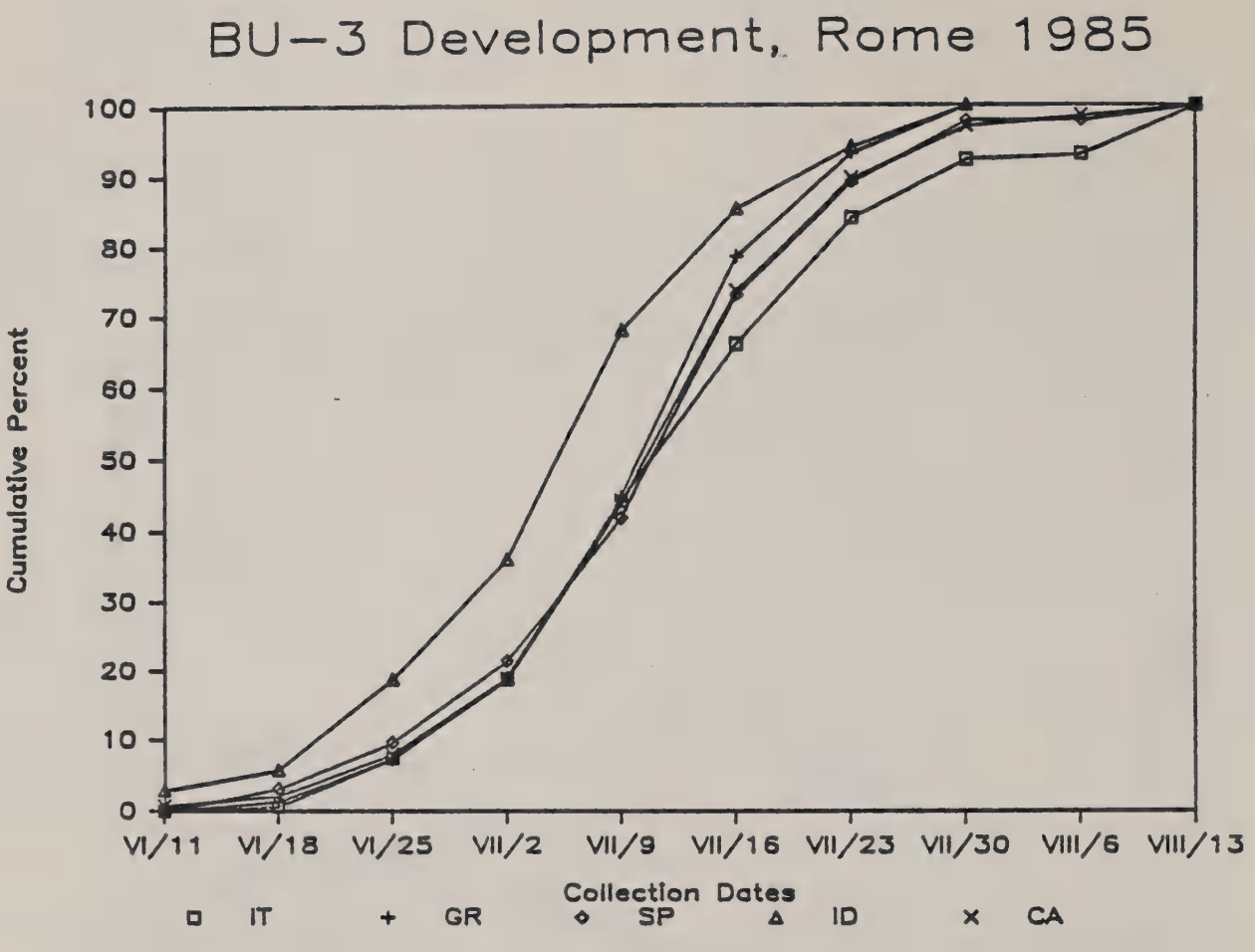


Fig. 2 : The percentage of insect damaged capitula from plants of five strains of Centaurea solstitialis L., July 9 through late season (August 27 - September 3), weed garden-plot study, Rome, Italy 1985 .



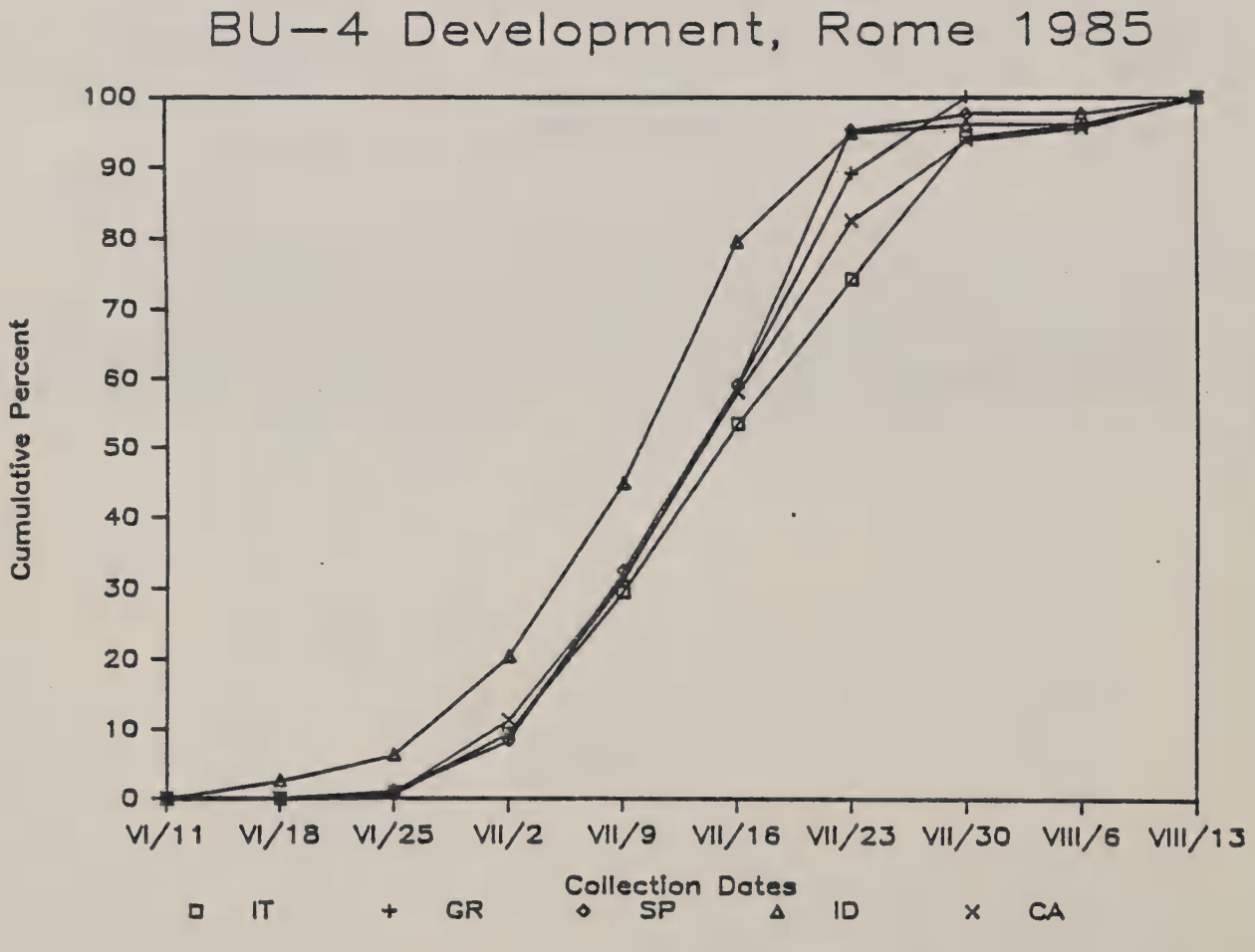


Fig. 3 : Bu-3 and Bu-4 bud development on plants of five strains of Centaurea solstitialis L. expressed as cumulative percent of the total counted for the season, weed garden-plot study, Rome, Italy 1985 .

Table 5. Measurements ($\bar{x}$+SE) of width and height of mature plants of different strains of Centaurea solstitialis L. (Asteraceae), and the number ($\bar{x}$+SE) of flowerheads produced in a season by plants of each strain, 1984 and 1985 garden plots, Rome, Italy.

Source of seed of the strains of Centaurea solstistialis	Mature plant width (cm)	Mature plant height (cm)	No. of flowerheads
	1984 Data, $\bar{x}$+SE (n=8)		
Sacramento, California	111.00 ± 7.47	95.38 ± 6.38 a 1/	738.63 ± 78.82a 1/
Lapwai, Idaho	95.38 ± 6.56	91.37 ± 5.55ab	338.64 ± 49.00bc
Contra Costa County, California	97.50 ± 9.38	85.88 ± 5.94abc	417.25 ± 41.88bc
Walla Walla, Washington	98.25 ± 5.26	80.25 ± 5.01abc	285.00 ± 30.62c
Rome, Italy	93.00 ± 3.60	75.38 ± 1.94bc	339.38 ± 64.02bc
Southern Spain	80.25 ± 4.57	71.88 ± 4.12c	566.00 ± 57.69ab
Yakima, Washington	78.50 ± 5.41	71.14 ± 2.97c	573.50 ± 123.41ab
Calculated F-ratio			
Blocks	1.06 NS	0.69 NS	1.99 NS
Treatments	1.45 NS	3.61, P < 0.01	6.41, P<0.01
	1985 Data, $\bar{x}$+SE (n=4)		
Sacramento, California	100.00 ± 11.41	82.75 ± 6.79a 2/	541.00 ± 101.42
Lapwai, Idaho	95.75 ± 5.59	99.75 ± 11.45a	392.75 ± 65.82
Rome, Italy	84.75 ± 13.76	62.75 ± 4.41b	561.50 ± 164.71
Southern Spain	108.75 ± 4.59	80.75 ± 3.20a	793.33 ± 135.75 3/
Thermi, Greece	91.00 ± 5.92	87.00 ± 1.68a	531.50 ± 49.14
Calculated F-ratio			
Blocks	3.34 NS	0.41 NS	4/
Treatments	1.69 NS	3.74, P < 0.05	1.52 NS

1/ Means followed by the same letter are not significantly different at the 0.01 level of significance (DMRT).

2/ Means followed by the same letter are not significantly different at the 0.05 level of significance (DMRT).

3/ Mean (±SE) is based on 3 plants.

4/ Analyzed as a single classification ANOVA, hence there is no F-ratio for block effects.

Thermi Garden-Plot

Species Diversity and Relative Abundance: The taxonomic composition and relative abundance of the insects that emerged from the capitula of the test plants in Thermi is shown in Table 6. The results show that U.S. YST plants are suitable hosts for Chaetorellia hexachaeta australis (Hering), Urophora sirunaseva (Hering), Terellia n. sp., Eustenopus hirtus (Waltl), Larinus curtus Hochhut, Bangasternus orientalis Cap., and Bruchidius tuberculatus (Hochhut). Bruchidius has been questioned as a potential candidate agent, and the suitability of U.S. YST as a host plant for U. sirunaseva and B. orientalis was already known (see previous Annual Reports).

The data in Table 6 reflect insect emergence from YST and Cirsium creticum during the summer and fall months. Overwintering emergence data is included for Carthamus tinctorius but not for Cynara scolymus because data from the latter was not tabulated at the time this report was written. Suffice it to say that none of the potential candidates emerged from Cynara. Also, only overwintering or developing larvae of Urophora sp. (probably stylata) and Lasioderma spp. were discovered when heads of C. creticum were dissected in winter 1986.

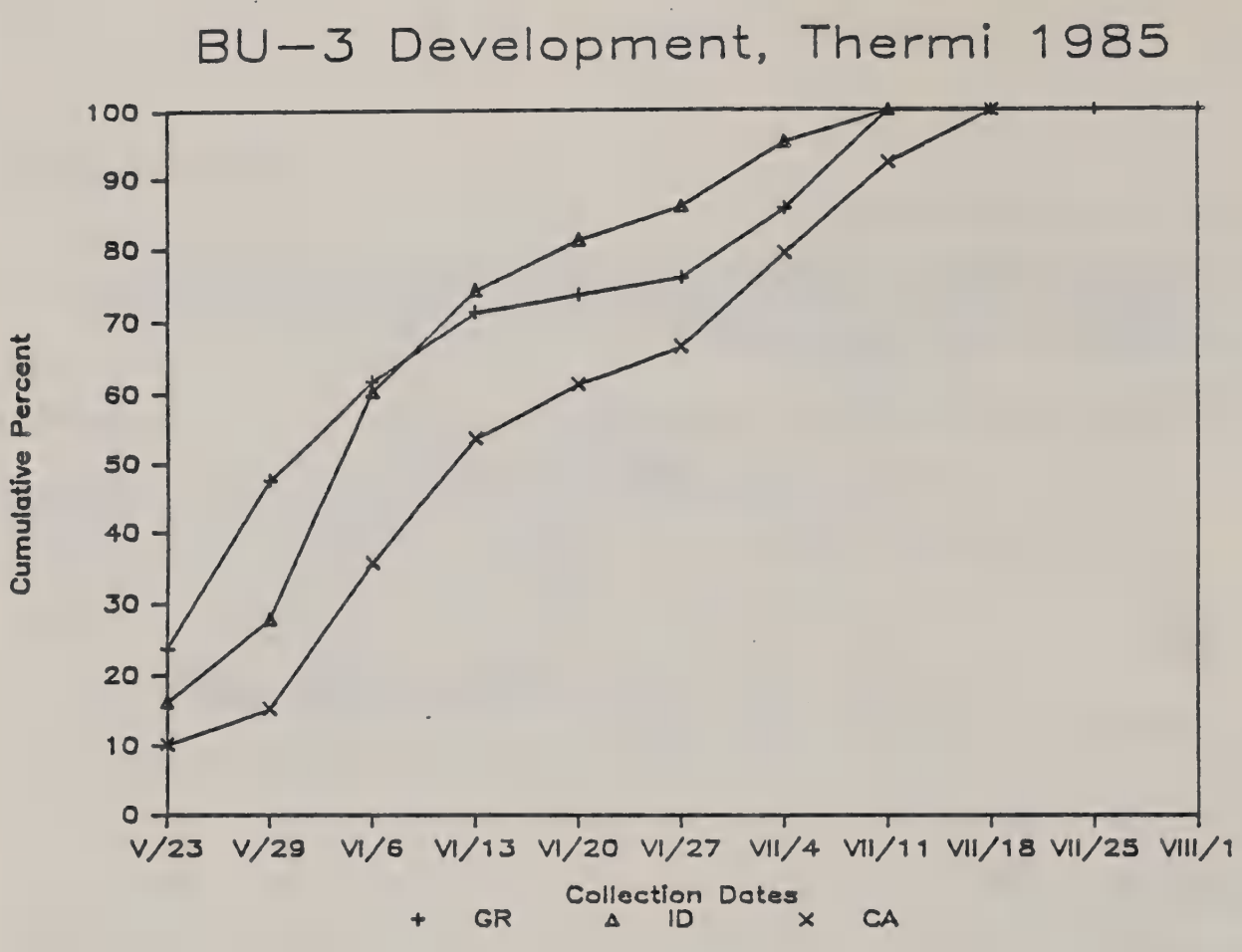
Relative to quantitative aspects of our dissections, the only information available at this time is data on the percentage of U. sirunaseva galls in capitula of the three YST strains. Galls were recovered in the samples collected between June 22 and July 4 with the following results: $5.63 \pm 1.70\%$ ($n = 6$ plants) and $2.92 \pm 0.95\%$ of the capitula from the Italian and Idaho plants, respectively, harbored galls. These values are not significantly different (calculated F ratio = 1.72 for transformed data; $P < 0.05$). During the same time period only two of five California plants had galls of U. sirunaseva, with 5.10% ($n = 98$ capitula) and 25.0% ($n = 12$) of the flowerheads of these plants harboring galls. The lower number of galls on the

Table 6 : Taxonomic composition of the insects that emerged from capitula of three strains of Centaurea solstitialis L. (YST), one cultivar of Carthamus tinctorius L., Cynara scolymus L., and Cirsium creticum (Lam.) D'Urv., Thermi, Greece 1985 .

	Number of Emerging Insects						
	Host Plants						
Insect Taxa	YST Thermi, Greece	YST Lapwai, Idaho	YST Sacramento, California	Carthamus tinctorius	Cirsium creticum	Cynara scolymus	TOTAL
Diptera: Teprhitidae							
Chaetorellia hexachaeta australis (Hering)	29	34	21	0	0	0	84
Acanthiophilus helianthi (Rossi)	0	14	4	281	0	4	303
Urophora sirunaseva (Hering)	17	13	5	0	0	0	35
Urophora stylata (Fabricius) *	0	0	0	0	13	0	13
Terellia n.sp.	13	4	7	0	0	0	24
Terellia fuscicornis (Lw.) *	0	0	0	0	0	419	419
Coleoptera: Curculionidae							
Eustenopus hirtus (Waltl.)	84	140	79	0	0	0	303
Bangasternus orientalis Cap.	0	2	1	0	0	0	3
Larinus curtus Hochhut	0	2	0	0	0	0	2
Larinus syriacus Schohlen	0	0	0	21	0	0	21
Larinus turbinatus Gyll.	0	0	0	0	4	0	4
: Anobiidae							
Lasioderma spp.	76	159	81	95	15	288	714
: Bruchidae							
Bruchidius tuberculatus (Hochhut)	25	68	66	0	0	0	159
Lepidoptera (unidentified)	0	0	0	3	6	2	11
Hymenoptera: Cynipidae							
Isocolus sp. * *	-	-	-	-	-	-	-
TOTAL	244	436	264	400	38	713	2095

* Tentative identifications .

* * Number of emerging Isocolus has not been tabulated .



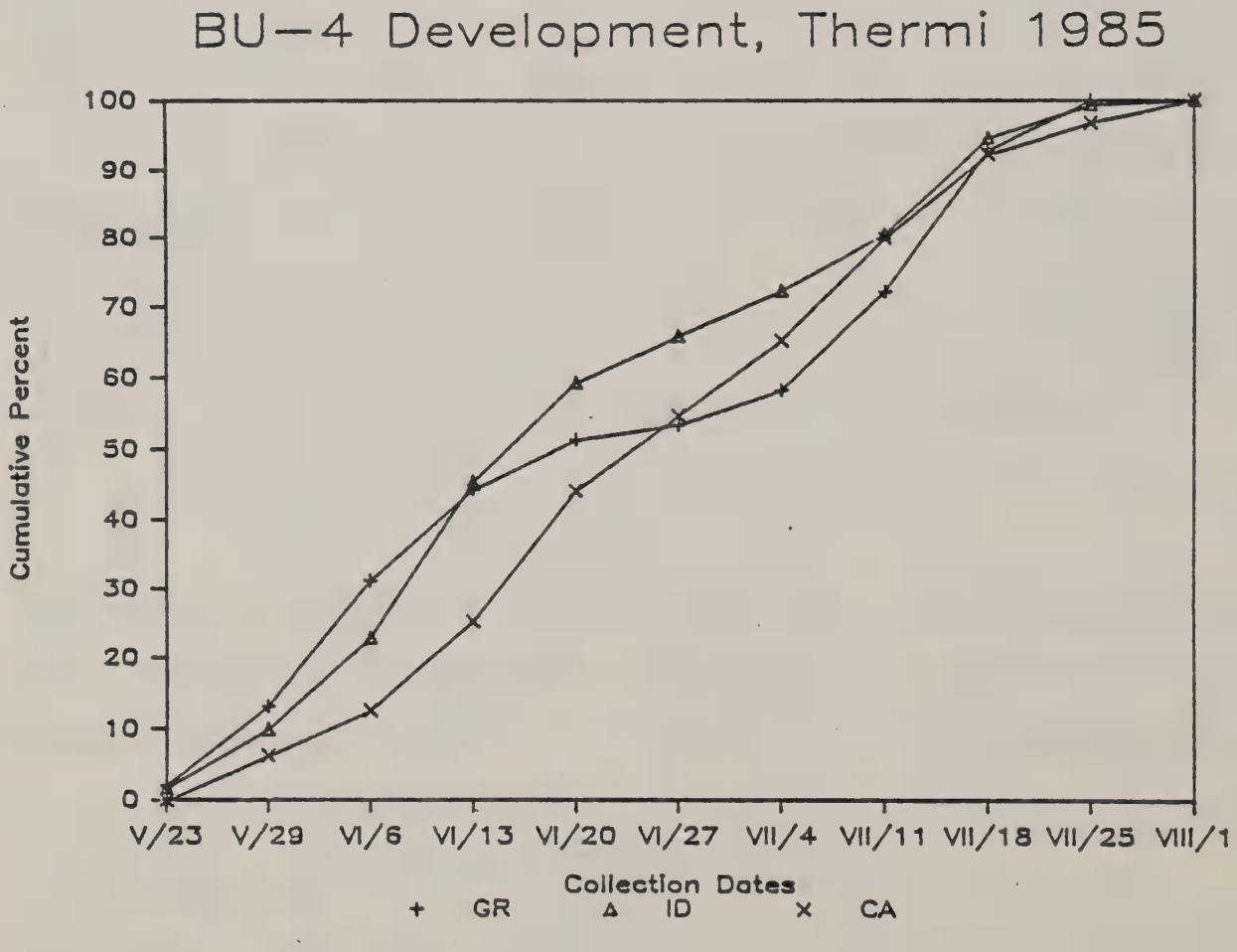


Fig. 4 : Bu-3 and Bu-4 bud development on plants of three strains of Centaurea solstitialis L. expressed as cumulative percent of the total counted for the season, weed garden-plot study, Thermi, Greece 1985 .

California plants may be related to phenotypic plasticity in bud development among the three strains (see Figure 4 which shows the delayed development of Bu-3 and -4 buds on California plants), rather than Urophora responding to a gradient of host plant suitability among YST "genotypes". Overwintering galls were found in four seedheads collected on two Idaho plants and one Greek plant between July 25 and August 1.

We acknowledge the following specialists for identifying the insects listed in Table 6. Dr. I. White (CIE, London) for Chaetorellia hexachaeta australis, Urophora sirunaseva and Terellia n. sp.; Dott. Enzo Colonnelli (Dipartimento di Biologia Animale e dell'Uomo, Viale dell'Università 32, Roma) for Eustenopus hirtus, Bangasternus orientalis, Larinus curtus, L. syriacus, and L. turbinatus; and Dr. R.E. White (Research Entomologist, Systematic Entomology Laboratory, USDA) for Lasioderma spp. Dr. White identified two species of Lasioderma from the material we submitted, one "near redtenbacheri" Bach and one "sp.", but we made no attempt to segregate the two species. The identities of Acanthiophilus helianthi and Bruchidius tuberculatus were checked by comparisons with specimens previously identifed by Dr. R.H. Foote (Research Entomologist, Systematic Entomology Laboratory, USDA) and Dr. M.L. Cox (CIE, London).

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Eustenopus hirtus (Waltl, 1838)

(Coleoptera: Curculionidae)

S. L. Clement and T. Mimmocchi

Eustenopus hirtus (Waltl, 1838) was chosen as a potential candidate biocontrol agent of Centaurea solstitialis L. (YST) after a literature review and information from curculionid specialists indicated the insect is closely associated with YST in the field, and with the knowledge that female beetle in Dr. Sobhian's 1984 studies failed to oviposit on cultivated safflower and demonstrated a need to feed on YST to develop eggs. We know that Sobhian and Zwölfer (1985) disqualified this insect as a candidate, primarily because "young.....larvae transferred from YST to safflower heads fed there normally". However, we believe that the present, over all level of understanding of the insect's biology, behavior and host plant range is insufficient to objectively determine its worth as a biocontrol agent.

The work program for 1985 was planned with the following objectives in mind: (1) to study the insect's oviposition and feeding behavior on various test plant species in the laboratory; (2) to collect more data on larval development in safflower buds; (3) to conduct an open field test in Thermi, Greece to determine the ability of the weevil to find and develop on different strains of YST, Cirsium creticum, Carthamus tinctorius and Cynara scolymus; (4) to survey various thistle plants in Greece and other parts of Europe to record the presence-absence of Eustenopus on these plants.

Objective 4 is part of a much larger study by Turner (USDA, ARS; Albany, California) and Sobhian and they will discuss their results elsewhere. Preliminary results from the open field test (objective 3) are discussed in another section of this Annual Report. This section of the report will focus mostly on objectives 1 and 2.

Taxonomy

Genus Eustenopus Petri

Eustenopus hirtus (Waltl, 1838)

Type species and Syn.: Larinus villosus Boheman.

Csiki (1934) recognized a large number of subgenera in the genus Larinus, and villosus was placed in the subgenus Eustenopus Petri. This is the currently accepted classification; however, many curculionid specialists (M.E. Ter-Minasyan (U.S.S.R.), E. Colonnelli (Italy), M.L. Fremuth (Czechoslovakia), M.L. Cox (U.K.), D. Whitehead (U.S.A.)) believe Eustenopus should be elevated to generic level. Besides E. villosus (=hirtus), Ter-Minasyan (1967) recognized two other species, namely E. lanuginosus Faust. and E. abbreviatus Faust.

The insect we are working with is E. hirtus (det. Dott. E. Colonnelli, Dipartimento di Biologia Animale e dell'Uomo, Viale dell'Universita' 32, Roma, Italia). The insect called E. abbreviatus in previous reports by USDA Rome and Thermi, Greece scientists is in fact E. hirtus. Centaurea solstitialis is the only known breeding host for this insect in its native range, which extends east from Greece to Iran and north into the Transcaucasus and parts of Central Asia. One can obtain a picture of the insect's distribution by looking at Fig. 1, which shows some of the sites where S. Clement, P. Dunn, and R. Sobhian have collected the insect.

METHODS AND RESULTS

Objective 1: Adult feeding and oviposition behavior.

A small number (n=27) of adult beetles were reared-out from YST heads collected during an August 1984 survey in central Greece. From this series, however, only 8 (5 ♂; 3 ♀) survived and these were used in attempt to learn more about the insect's feeding and oviposition on YST. These eight beetles

were set up with YST rosettes on March 5, after which they were offered a progression of host plant growth stages through June 10. Details of this study are provided in Table 1, which also shows the extent of feeding, mating and oviposition behavior in relation to YST growth stages.

Sobhian and Clement have observed adult feeding on Bu-1 buds and oviposition in Bu-4 buds in the field in Greece. Similar behavior was observed in the laboratory (Table 1).

During the last week of June, R. Sobhian and S. Clement collected 257 beetles in a patch (ca. 0.5 hectare) of YST on the southern outskirts of Thessaloniki and another 72 on YST along the road ca. 2 km. south of Doirani, Greece. Mortality during transit to Rome was fairly high (n=54), which left 275 for a no-choice feeding and oviposition test. Beetles were allowed to feed on buds of YST for 48 hours before mating pairs were selected for the no-choice test (June 29-July 24). The test plants and experimental details are given in Table 2. A second collection of beetles was made by R. Sobhian in July but less than 10 survived the trip to Rome.

Adults fed on the capitula of all the test plant species and strains of YST tested (Table 2) causing considerable damage to the capitula of YST, Centaurea nicaeensis and C.diffusa, Cnicus benedictus, and Cirsium spp. It is important to stress, however, that eggs were only deposited into the buds of Centaurea spp. Adult survival at the end of the test was good (>40%) on Centaurea spp., Cirsium arvensis, C. douglasii and fair (8.3 and 20%) on Carthamus tinctorius and C. benedictus. Beetle mortality was 100% on the remaining test plant species .

Another study was done to observe beetle feeding and oviposition in buds of YST and C. tinctorius in small containers (500-cc cups) under the same quarantine conditions. The beetles were drawn from those used to measure oviposition on YST in the no-choice test. One cup held 10 beetles and a bouquet of 5 (stage Bu 3-4 YST) buds; the other cup held 10 beetles and a bouquet of 3 closed buds of C. tinctorius. Every 3-5 days fresh bouquets were provided and the old buds were examined for feeding punctures and eggs . At the same time, dead weevils were counted, then removed from the cups and dissected to examine the condition of the ovaries in females.

The beetles lived an average of 14.7 ± 6.85 ($\bar{x}$ ± SD) days on YST and 9.90 ± 7.23 days on safflower in the 500 cc cups. These averages are not significantly different ($P < 0.05$; t-test). The 3 females in the YST cup laid 35 eggs between July 11 and August 8. No eggs were laid by the 4 females in the safflower cup between July 11 and July 28. One egg was discovered in the ovaries of a female from the safflower cup when she was dissected. Six females did not have eggs in their ovaries when they died. The beetles punctured the safflower buds but they were not riddled with holes like the YST buds.

Objective 2 Larval development in safflower buds.

Not much was accomplished under this objective. Table 3 shows that some of the neonate larvae placed in buds of potted safflower plants fed and molted.

Miscellaneous Data: The average (± SD) length and width of 17 newly laid eggs was, respectively, 1.11 ± 0.08 mm and 0.63 ± 0.05 mm. The head capsules of 27 larvae randomly extracted from buds on potted YST plants in the quarantine greenhouse in July were measured . The measurements (mm) were: 0.46 (n=3); 0.48 (n=7); 0.50 (n=3); 0.52 (n=2); 0.68 (n=1; recently molted); 0.70 (n=1); 0.88 (n=1); 0.96 (n=1); 1.10 (n=1); 1.16 (n=1); 1.20 (n=2); 1.22 (n=1); 1.24 (n=1); and 1.28 (n=1).

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Table 1. Observations on Eustenopus hirtus adult feeding, mating and oviposition on a progression of host plant growth stages of Centaurea solstitialis, laboratory study, Rome, Italy, 1985.

	DATES OF OBSERVATION			
	March 5 - April 28	April 29 - May 7	May 8 - June 3	June 4 - 10
	PLANT STAGE OFFERED TO BEETLES			
Activity Observed	Rosettes 2/	Initial bolt phase 2/	Floral bud stage 3/ 4/ (Bu-1)	Floral bud stage 3/ 5/ (Bu-1 to 4)
Feeding:	No	Minor stem and leaf midrib feeding	Extensive feeding on Bu-1's and minor feeding on stems	Extensive feeding on all bud stages
Mating:	No	No	No	Yes, on Bu 3-4 buds
Oviposition:	No	No	No	Yes, in Bu-4 buds

1/ Study was conducted in a laboratory under temperatures of 18-33°C, RH of 35-85% and natural light.

2/ YST plants in these stages were offered to beetles in 500 cc. cardboard containers (2 containers each with 4 beetles) with organdy cloth lids. Plants were removed from the laboratory garden and held in water-filled vials. Plants were changed twice per week.

3/ YST plants in these more advanced stages were offered to beetles as potted plants. A plastic-cylinder cage with an organdy cloth lid was placed over the plant.

4/ The eight beetles were caged with one potted plant; they were moved to a new plant on May 22.

5/ Beetles were coupled with one potted plant from June 4-10.

Table 2. Results of no-choice oviposition studies with Eustenopus hirtus (Waltl), June 29 to July 24, 1985, Rome, Italy[1].

Test Plant	Source of Test Plant	Plant No.	No. Beetles ♀	No. Beetles ♂	No. Closed Buds	No. of Feeding Punctures on Buds	Duration of Test (days)	No. Beetles Alive After Test	No. Eggs(E) or Larvae(L) Found at Dissection
1. Centaurea solstitialis	Thermi, GR.	1	1	1	8	too numerous to count on all buds[3]	9	1♀	7E; 3 neonate L
		2	1	1	8	"	9	2	4E; 2 neonate L
		3	1	1	4	"	6	2	3E
		4	1	1	6	"	6	2	4E; 1 neonate L
		5A[2]	1	1	8	"	10	1♀	3E; 3 1L[6]
		5B	1	1	3	"	10	0	3 1 L
		6	1	1	5	"	8	2	3E; 1 neonate L
		7	1	1	4	"	7	0	3E; 1 1L
		5C	3	3	9	"	7	1♀	5E; 4 1L
		[illegible]	?	?(n=4)	5	"	7	4[5]	7E
		9	?	?(n=6)	9	"	8	4[5]	6E;2 1L
2. Centaurea solstitialis	Walla Walla, WA	1	1	1	5	"	11	2	3 1L
		2	1	1	4	"	10	1♀	3E; 1 1L
		3	1	1	9	4[4]	10	1♂	0
		4	?	?(n=6)	5	"	19	5[5]	2 late-instar larvae; 1 prepupa
3. Centaurea solstitialis	Yakima, WA.	1	?	?(n=11)	ca. 30	too numerous to count	6	11[5]	Three larvae found July 24 in 3 F2 flowerheads
4. Centaurea nicaeensis	San Severo IT	1	1	1	6	4	4	1♀	2E
		2	1	1	3	2	4	0	0
		3	1	1	8	14	7	2	9E; 2 1L
5. Centaurea diffusa	Greece	1	1	1	47	too numerous to count	8	2	0
		2	1	1	81	"	8	1♀	2 dead neonate larvae
		3	1	1	38	"	7	0	0
		4	1	1	26	"	7	0	0
		5	1	1	11	"	7	1♀	0

Table 2 cont.d.

Test Plant	Source of Test Plant	Plant No.	No. Beetles ♀	No. Beetles ♂	No. Closed Buds	No.of Feeding Punctures in Buds	Duration of Test (days)	No. Beetles Alive After Test	No.Eggs(E) or Larvae(L) Found at Dissection
6. Carthamus tinctorius	U.S. var.Hartmen	1	1	1	6	2	7	0	0
		2	1	1	4	6	7	0	0
		3A	1	1	4	7	7	0	0
		3B	1	1	3	0	7	0	0
		4A	1	1	4	2	8	0	0
		4B	1	1	3	0	4	0	0
		5	1	1	4	3	3	0	0
		6	1	1	4	4	4	0	0
		7	1	1	4	2	4	0	0
		8	2	4	5	6	5	0	0
		9	3	3	3	1	5	3	0
		10	4	2	5	6	5	0	0
7. Carthamus lanatus	Thermi,GR.	1A	1	1	8	0	7	0	0
		1B	1	1	6	0	4	0	0
		2	1	1	9	1	7	0	0
		3	1	1	8	0	7	0	0
		4	1	2	7	5	7	0	0
		5A	1	1	11	0	4	0	0
		5B	2	4	8	0	5	0	0
8. Carthamus dentatus	Thermi,GR.	1A	1	1	5	0	4	0	0
		1B	4	2	9	0	5	0	0
		2	1	1	7	1	4	0	0
		3	1	1	4	0	6	0	0
		4A	1	1	6	0	4	0	0
		4B	3	3	8	0	5	0	0
		5	1	1	4	0	4	0	0
9. Cynara scolymus	U.S.	1	4	5	1	2	5	0	0
10. Helianthus annuus	U.S. var. Parendovik	1	1	1	4	0	7	0	0
		2	1	1	3	0	4	0	0
		3	1	1	3	0	4	0	0

Table 2 cont.d.

Test Plant	Source of Test Plant	Plant No.	No. Beetles ♀	No. Beetles ♂	No. Closed Buds	No. of Feeding Punctures in Buds	Duration of Test (days)	No. Beetles Alive After Test	No. Eggs(E) or Larvae(L) Found at Dissection
11. Lactuca sativa	U.S. var. Bibb. (Limestone)	1	1	1	53	6	5	0	0
		2	1	1	41	11	5	0	0
		3	1	1	47	2	5	0	0
12. Cnicus benedictus	Trieste Botanical Garden, IT.	1	1	1	4	too numerous to count	8	2	0
		2	1	1	4	"	8	0	0
		3	1	1	5	"	8	0	0
		4	1	1	4	"	8	0	0
		5	1	1	4	0	8	0	0
13. Cirsium arvensis	Rome, IT.	1	1	1	15	2 buds riddled with punctures	4	0	0
		2	1	1	13	2	4	2	0
		3	1	1	12	4 buds riddled with punctures	8	2	0
		4	1	1	16	2 buds riddled with punctures	4	0	0
		5	1	1	21	5 buds riddled with punctures	7	0	0
14. Cirsium undulatum	U.S., CA.	1A	2	2	4	3	4	0	0
		1B	2	2	4	1 bud riddled with punctures	4	0	0
		2	2	2	7	too numerous to count	4	0	0
15. Cirsium douglasii	U.S., CA.	1	3	3	7	too numerous to count	5	3♀, 2♂	0

1/ With the exception of the Cynara scolymus - beetle test, which was started on July 17, all other plant-beetle tests were set-up between June 29 and July 3. This test was conducted in the Laboratory quarantine (18 - 33° C; RH 35-85%; natural light).
2/ Separate nylon tulle cages were placed over two to three branches on some plants, hence the A, B, or C designations with some plant numbers.
3/ The rating "too numerous to count on all buds" means that all or most of the buds were riddled with feeding punctures, making it impossible to distinguish and count single punctures.
4/ One number alone indicates the total number of punctures on the number of buds exposed to the beetles.
5/ Beetles were not dissected for sex determination because they were saved for additional studies.
6/ 1L = first instar larva.

Table 3. Results of a small-scale larval survival test with Eustenopus hirtus on Centaurea solstitialis and Carthamus tinctorius, Rome, Italy 1985.

Test Plant	Bud No.	Width (mm) of pre-flowering Buds 1/	Dates		Results at Dissection
			Infested	Dissected	
Centaurea solstitialis (Greek Plant)	1	9.0	July 11	July 25	Healthy late-instar larva
	2	8.5	July 16	July 25	Healthy half-grown larva
	3	8.0	July 13	July 25	No larva; evidence of past feeding.
Carthamus tinctorius (U.S. variety "Hartman")	1	12.5	July 16	July 25	Dead half-grown larva 2/
	2	11.0	July 17	July 25	Healthy half-grown larva 2/
	3	15.5	July 11	July 25	No larva; minor feeding damage
	4	12.0	July 17	July 25	Dead late-instar larva 2/
	5	9.0	July 15	July 25	No larva; minor feeding damage
	6	10.0	July 16	July 25	No larva; minor feeding damage

1/ Neonate larvae were transferred to small holes bored into the buds.

2/ Larvae inflicted noticeable damage to the flowerheads.

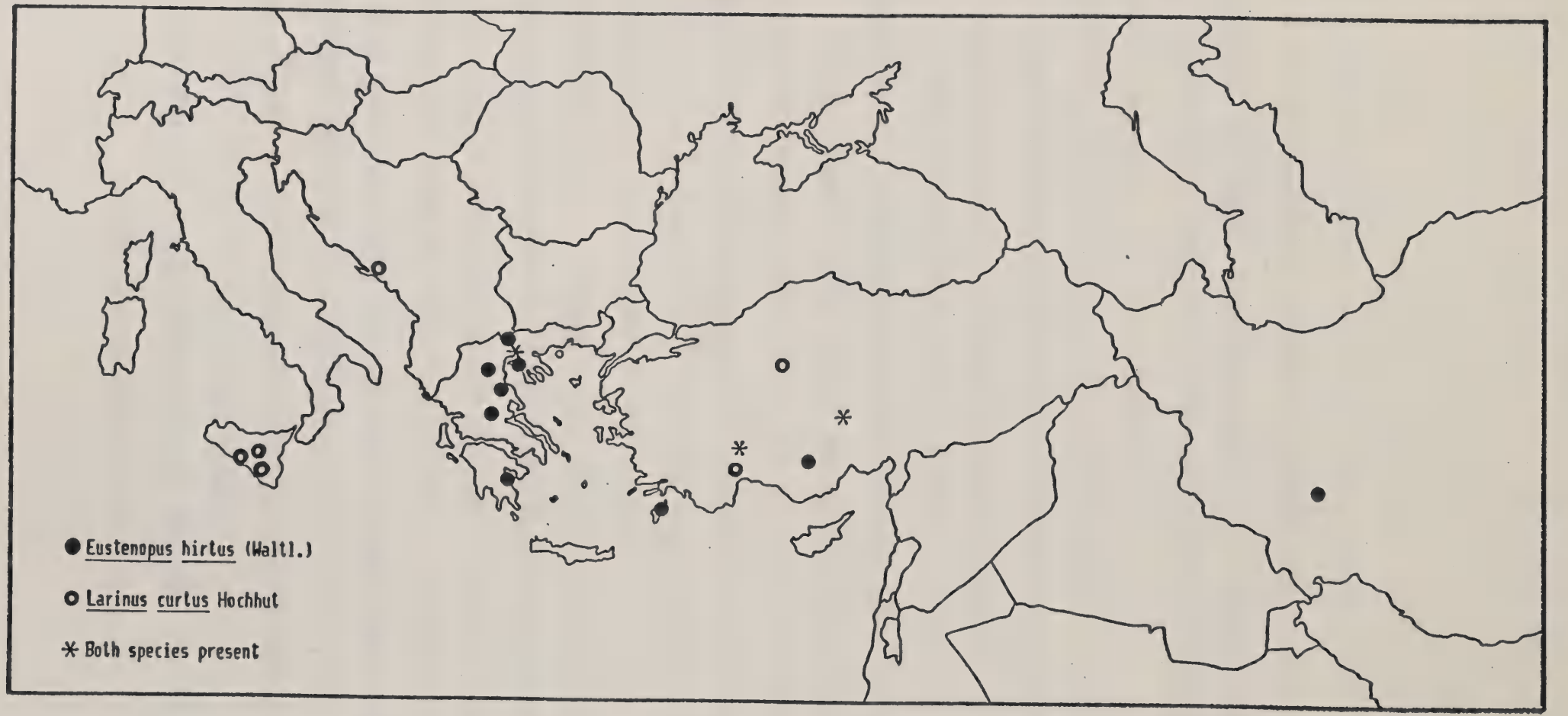

Fig. 1 : Sites shown are where S. Clement, P. Dunn and R. Sobhian have collected Eustenopus hirtus . The sites for Larinus curtus were obtained from labels on a series of museum specimens identified by E. Colonnelli and from the personal collection records of P. Dunn and S. Clement .

Larinus (*Larinomesius*) *curtus* Hochhut

(Coleoptera: Curculionidae)

Clement and Mimmocchi

We decided to conduct laboratory host specificity studies with *Larinus* (*Larinomesius*) *curtus* Hochhut when beetles (n=31) were collected on F-2 YST flowerheads (see Maddox 1981 for description of plant stages) on June 23 and 24 near Thessaloniki, Greece. The identity of this species was established by Dr. E. Colonnelli, Zoology Department, University of Rome, Italy. The abdominal setae are deeply split on *L. curtus* and this characteristic is used to distinguish it from the closely related species *obtusus* Gyll., *australis* Cap., *minutus* Gyll., and *canescens* Gyll. (Zwolfer et al. 1970).

We have not disqualified this insect as a candidate biocontrol agent of YST despite Sobhian and Zwölfer's (1985) statement that the adults exhibit a family broad feeding spectrum under artificial laboratory conditions. In the field, the beetle appears to be closely associated with *Centaurea solstitialis* L. (Zwölfer et al 1970) and *C. calcitrapa* (Fremuth 1982). Moreover, "pollen feeding is possibly a prerequisite for oogenesis, as females concentrate on open flowerheads" of YST in the field (Sobhian and Zwölfer 1985). Also, a review of the literature indicated that *L. curtus* is not a known pest of artichoke or cultivated safflower; however, two species in the genus *Larinus* are pests of artichoke, namely *L. cynarae* E. and *L. scolymi* Oliv. (Prota 1985). These positive points provide justification for expanded host range, adult feeding, and female oogenesis studies of *L. curtus*. The small-scale study described herein was undertaken in an attempt to learn more about these aspects.

Methods and Results

A no-choice oviposition test was conducted between July 7 and August 12 in the quarantine facility at the Rome Laboratory. In the quarantine, maximum and minimum daily temperatures were, respectively, 29-36°C and 16-22°C; lighting was natural and RH was 28-85%. Test plants, the number of plant replicates and beetles tested per plant, and other experimental details are shown with the results in Table 1. Beetles were placed in nylon tulle (black) cages which were placed over branches of flowering test plants.

Beetles were kept for 4-6 days on a flowering YST plant from Greece before they were used in the test. Beetles were reproductively active and females laid eggs during this pre-test period. Seven of the 31 beetles were reproductively active until they died on August 18 and 19.

Pollen feeding by adults resulted in damage to the florets of the test plants (Table 1). Female beetles laid eggs in the open flowerheads and larvae fed and developed in the heads of YST from Greece (control plants), Rome, Italy, Sacramento, California, and Yakima, Washington (Table 1). One to three eggs were found in a flowerhead; however, only one larva completed its development to an adult in four YST heads where 2 or 3 viable eggs were laid. The achenes in these four heads were all destroyed by the feeding larvae. Each egg was protected by a thin-shelled capsule, which was "glued" to several florets.

Two males and two females were transferred to a flowering YST (Rome, Italy) plant after they had fed in the open flowers of Carthamus tinctorus for 22 days. At least one of the two females laid viable eggs on the YST plant (Table 1), so some beetles can live for a fairly long time on a "non-host plant", albeit in captivity, and resume reproductive functions once back on the normal host. Three females were dissected within 48 hours after they had

died on safflower and no eggs were found in their ovaries. Captive adults were observed copulating repeatedly on YST, C. nicaeensis, and Carthamus tinctorius. A mating pair was collected on a YST flowerhead (F-2 stage) at the Thessaloniki, Greece collection site on June 24.

A pair of beetles (1 ♀, 1 ♂) was exposed to a flowering Yakima YST plant for 11 days (plant IB in Table 3) before being transferred on August 2 to a Rome YST plant that had been exposed first to flies of Chaetorellia sp. nr. carthami Stack (Diptera: Tephritidae) in the Laboratory garden. On the Rome plant the female beetle was exposed to 21 flowerheads (F-2 stage) over a 16-day period before she died on August 18. Dissections of the YST heads (seed formation stage) on 22 August revealed the following:

(1) 17 eggs were laid during the 16-day period; two heads had 2 eggs each, while 13 had one egg. Eggs were not laid in 6 flowerheads.

(2) Eggs were laid in 4 heads harboring larvae of Chaetorellia. The beetle eggs hatched and some larval feeding took place but they died before reaching the 3rd instar. It appeared that the fly larvae had devoured most of the intact achenes; therefore, not enough food was left for the beetle larvae to complete their development. The tephritid fly inserts eggs into Bu-4 buds and the larvae become prepupae by the time the flower has reached the seed formation stage.

(3) The presence of a new pupa of L. curtus in one head indicated that development from egg (if laid on August 2) to pupa occurred over a 20-21 day period. In another instance (see Table 1), it took 19 days for development from egg to pupa.

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Table 1. Results of no-choice oviposition studies with Larinus curtus Hochhut, July 7 to August 12, 1985, Rome, Italy.

Test Plant	Source of Test Plant	Plant No. [1]	No. Beetles ♂	No. Beetles ♀	No. Flowerheads	Duration of Test(days)	No. Beetles Alive After Test	Results from Dissections No. Eggs(E); and Larvae (L); Extent of Adult Feeding on Flowers [2]
1. Centaurea solstitialis	Thermi, GR.	1.	1	1	5	9	1♀	5 E; 3 1L [6]; slight
		2.	1	1	3	9	2	0 E; 0 L; slight
		3.	1	1	5	9	2	1 E; 1 1L; slight
		4.	1	1	3	8	1 ♂	4 E; 3 1L; slight
2. Centaurea nicaeensis	San Severo, IT.	1.	1	1	3	6	2	0 E; 0 L; slight to extensive
3. Carthamus tinctorius	U.S."Hartman" Cultivar	1.	1	1	4	9	0	0 E; 0 L; slight
		2.	2	2	5	9	0	0 E; 0 L; slight to moderate
		3.	5	3	14	22	2♂, 2♀ [3]	0 E; 0 L; slight to extensive
4. Centaurea solstitialis	Sacramento, CA.	1A	1	1	10	19	2 [4]	3 E; 1 1L and 1 late-instar larva; slight
		1B	?	?(n=5)	5	3	5 [5]	2 E; 0 L; none
5. Centaurea solstitialis	Yakima, WA.	1A	?	?(n=5)	12	14	5	4 E; 1 Pupa 19 days after start of test; none to slight
		1B	1	1	6	11	2	4 E; 0 L; none
6. Centaurea solstitialis	Rome, IT.	1.	2	2	9	18	1♀, 1♂	5 E; 2 half-grown larvae; slight

[1] Numbers 1A and 1B stand for two branches fitted with gauze sleeve cages one plant.
[2] Adult flower feeding was rated as none, slight, moderate, or extensive.
[3] These 4 adults were transferred to Test Plant #6 (C. solstitialis from Rome, IT.).
[4] This pair of adults were transferred to Test Plant 5-1B (C. solstitialis from Yakima, WA.).
[5] These 5 adults (sex ratio unknown) were transferred to Test Plant 5-1A (C. solstitialis from Yakima, WA.).
[6] 1L = first instar larva.

Chaetorellia hexachaeta australis Hering

(Diptera: Tephritidae)

Mimmocchi & Clement

The tephritid fly *Chaetorellia hexachaeta australis* Hering (called *C. hexachaeta* in previous reports) was earmarked at the beginning of the 1985 research season for expanded host plant specificity and biological studies. This decision was made because previous work had shown the fly to be restricted, in the field, to a few species of *Centaurea* (Sohbian and Zwölfer 1985) and Dr. Sohbian's unpublished studies in Greece showed the fly had a strong preference for ovipositing on buds of *Centaurea solstitialis* L. (YST) under laboratory conditions. Moreover, it was felt that this insect would complement the control action of another seed eating insect, the weevil *Bangasternus orientalis* Cap., already released in western U.S.

The taxonomy of *Chaetorellia* must be straightened-out before any species in the genus can become a candidate biocontrol agent. This is because a congeneric, *C. carthami* Stackelberg, is a known pest of cultivated safflower (e.g. Al-Ali et al. 1979) and the taxonomic position of several *Chaetorellia* associates of *Centaurea* spp. is unclear (Dr. I. White, C.I.E., pers. comm.). Also, Agriculture Canada is interested in a *Chaetorellia* associated with *C. maculosa* and related knapweeds in Europe. It is not known at the time of this writing if this *Chaetorellia* also breeds in YST in Greece.

The work program for 1985 had five objectives: 1) To study the fly's biology and seasonal activity patterns in northern Greece. Principal investigator was R. Sohbian. 2) To conduct an open field test in Thermi, Greece to determine the ability of the fly to find and develop on different strains of YST, and plants of *Cirsium creticum*, *Carthamus tinctorius* and

Cynara scolymus. Principal investigators were S. Clement and R. Sohbian.
3) To conduct no-choice oviposition tests in the quarantine laboratory in Rome, Italy. Principal investigators were T. Mimmocchi and S. Clement.
4) To survey various thistle plants in Greece and other parts of Europe to record the presence-absence of Chaetorellia on these plants. Principal investigator was C. Turner of the USDA, ARS Laboratory in Albany, California.
5) To study the behavior and reproductive biology of C. h. australis. Principal investigator was I. Pittara, a graduate student at the University of Thessaloniki, Greece.

Sohbian addresses various aspects of the fly's bionomics in another part of this Annual Report. Preliminary results of the open field test in Greece are also dicussed in another section. Turner (objective 4) and Pittara (objective 5) will report on their own research.

This short report will outline our attempts to fulfill Objective no. 3, the oviposition of the fly under no-choice conditions.

Experimental Procedures and Results

About 200 overwintering larvae of C. h. australis in YST heads were collected by R. Sohbian in Thermi, Greece between February 20 and March 29, 1985. This material was kept at 8°C in Thermi until it was shipped to Rome on May 8. In Rome, the flies were kept in a refrigerator (8-12°C; total darkness) in the quarantine laboratory.

The flies started to emerge in the refrigerator on May 15, which came as a surprise to us because buds in the right stage (Bu-3, Bu-4) were not yet available on the YST plants in nature. However, R. Sohbian discovered that C. h. australis emerges in the spring, ovipositing and completing a generation on C. cyanus before attacking and completing 1-2 subsequent generations on YST.

We used these early emerging flies for a preliminary oviposition test (Test 1), the results of which are summerized in Table 1. A larger no-choice oviposition test (Test 2) was conducted using flies that emerged from C. cyanus. Capitula of this Centaurea were collected by R. Sohbian and S. Clement on June 23-24 at two sites (Doirani and Agios Prodromos) in northern Greece and shipped to Rome where several hundred adults were reared out in the quarantine laboratory. In each study, test plants were pruned as needed and moved into the quarantine laboratory where a sleeve cage (made of black nylon mesh) was fitted over one branch per test plant and closed at both ends. Flies (2♀; 1♂ per cage in test 1; 3 couples per cage in test 2) were carefully introduced into cages through a small slit made in the cloth. Cardboard "nutrient strips" (yeast-hydrolysate, sucrose) were added to each cage. Water was provided via water filled vials with cotton plugs in test 1; in test 2, water was sprayed on the cages twice daily.

Test 1 was conducted under minimum daily temperatures of 14-20°C and maximum daily temperatures of 29-33°C, and minimum daily values of 30-40% RH and maximum values of 65-90%RH. In test 2, corresponding temperatures and RH values were 18-21°C, and 28-34°C, and 34-45% RH and 70-85% RH. Tests were conducted under natural light. The flies readily mated under these conditions.

Most of the flies in Test 1 died after a few days, thus the results in Table 1 are rather inconclusive. Fly longevity was also short in test 2, thus no conclusions can be drawn from this test either. Table 2 lists the test plants used in test 2.

It is apparent that we did not create optimal conditions for carrying out the tests . Perhaps daytime temperatures were too high for the flies, or nutrition was inadequate. Although mating was repeatedly observed, the flies were never seen resting or feeding on the nutrient strips.

CONCLUDING REMARKS

In December 1985 Clement talked to D. Maddox (USDA, ARS Albany Laboratory) and suggested that consideration be given to introducing C. h. australis into the Albany quarantine for expanded host specificity studies. This way, the bulk of the Chaetorellia testing could be done at Albany while Rome would focus on the biocontrol potential of Eustenopus hirtus (=villosus) and Larinus curtus, two seed eating weevils. This suggestion was accepted and D. Maddox wrote and submitted a petition to the Tecnical Advisory Group on Biological Weed Control.

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Table 1. Results of a no-choice oviposition test with *Chaetorellia hexachaeta australis*, Rome, Italy, May 28 - June 15, 1985.[a/]

Test Plant	Plant No.	Stage of Plant	Longevity (Days) ♀1, ♀2, ♂	No. of Eggs laid
Centaurea solstitialis (Thermi, Greece)	1	Bu 1-2-3-4[b/]	[c/]5, 6,(5)	0
	2	Bu 1-2-3-4	6, 6,(5)	1 on Bu 4
	3	Bu 1-2-3-4	3,10,(6)	3 on Bu 3 7 on Bu 4
	4	Bu 1-2-3-4	2, 5,(2)	0
	5	Bu 2-4	4, 4,(4)	0
	6	Bu 1-4	4, 4,(4)	0
	7	Bu 1-2-4	[d/] , ,(5)	3 on Bu 2 5 on Bu 3 14 on Bu 4 1 hatched la. in Bu 4
	8	Bu 1-2-4	4, 4,(4)	0
	9	Bu 1-2-4	5, 3,(5)	0
Centaurea cyanus	1	Buds, Flowers	6, 5,(10)	0
	2	Buds, Flowers	4, 4,(5)	0
	3	Buds, Flowers	3, 3,(3)	0
	4	Buds, Flowers	14[d/]15,(5)	0
Centaurea nicaeensis	1	Buds, Flowers	3, 5,(5)	0
	2	Buds, Flowers	4, 6,(5)	0
Galactites tomentosa	1	Buds, Flowers	3, 4,(4)	0
Carthamus tinctorius [e/] var. Hartman, US	1	Buds, Flowers	[d/] , ,(5)	0
	2	Buds, Flowers	2, 4,(4)	0
	3	Buds, Flowers	4, 2,(5)	0

a/ Test No. 1

b/ Buds classified according to Maddox, 1981.

c/ The first and second figures represent the number of days that females no.1 and 2 lived, respectively, while the third number ( ) is the number of days the single male lived.

d/ The same two females were tested against three test plants: first on *Carthamus tinctorius* (7 days), secondly on *C. solstitialis* (5 days), thirdly on *C. cyanus* until they died.

e/ Diameter of flowerheads was ca. 20-25 cm.

Table 2. List of test plants used in no-choice oviposition test, *Chaetorellia hexachaeta australis*, Rome, July 1985. [a]

Test Plant	Source of Seeds	No. of Replicates
Centaurea solstitialis	Thermi, Greece	12
C. solstitialis	Walla Walla, WA, USA	5
C. diffusa	Greece	7
C. calcitrapa	Puglia, Italy	5
C. nicaeensis	Puglia, Italy	4
C. cyanus	Trieste, Bot. Garden, Italy	3
Carlina sp.	Puglia Italy	4
Carthamus tinctorius	var. Hartman, USA	5
C. lanatus	Thermi, Greece	7
C. dentatus	Thermi, Greece	7
Galactites tomentosa [b]	Rome, Italy	4
Carduus nutans [b]	Rome, Italy	5
C. pycnocephalus	Trieste, Bot. Garden, Italy	7
Cirsium arvensis [b]	Rome, Italy	5
C. undulatum	CA, USA	1
Lactuca sativa	var. Bibb.(Limestone) USA	4
Cnicus benedictus	Trieste, Bot. Garden, Italy	5
Zinnia elegans	Trieste, Bot. Garden, Italy	7

[a] Test no. 2
[b] Plants collected in rosette stage in the field and transferred to pots.

Centaurea diffusa

INTRODUCTION

In this reporting period the work on *Centaurea diffusa* centered around 3 organisms: the moth *Pterolonche inspersa* Stgr., the weevil *Bangasternus fausti* Reitter and the eriophyid mite *Aceria centaureae*.

The *P. inspersa* work was directed towards finding infestations, making collections, and sending insects to Albany for final testing. We also provided Albany with a host-specificity testing data and information on the biology of the insect for inclusion in the petition for introduction.

Dott.ssa Marisa Castagnoli (Istituto Sperimentale per la Zoologia Agraria, Florence) provided us with the identification of the eriophyid mite (*Aceria centaureae* (Nal.)) which galls the leaves of diffuse knapweed rosettes in Greece. Also, we conceived the idea of growing certain U.S. *Cirsium* species in Greece so open field trials could be made with the eriophyid mite and eventually *Eustenopus hirtus*, described in the yellow starthistle part of this report. To this end, Dr. Byron Katsoyannos (Professor of Entomology, University of Thessaloniki, Thessaloniki, Greece) was invited to Rome to discuss a cooperative project, the cornerstone of which was growing plants of U.S. origin in Greece. Another object of the tests was to catalog any other indigenous insects or mites that feed on or used the American plants.

Pterolonche inspersa Staudinger

(Lep., Pterolonchidae)

Paul H. Dunn and Gaetano Campobasso.

A small root boring moth, *Pterolonche inspersa* (Staudinger), was tested as a potential biological control agent for Diffuse Knapweed (*Centaurea diffusa*). Sixty-two species of plants in six families were tested in 1st instar larval survival tests. Although occasional feeding occurred in some other *Centaurea* spp., *P. inspersa* has potential as a biocontrol agent against diffuse knapweed and its release in United States is recommended.

INTRODUCTION

Centaurea diffusa Lam., is an introduced weed of European origin that is spreading throughout the uncultivated, drylands of western Canada and northwestern United States. In the United States, it was first discovered in an alfalfa field in Bingen, Klitekat county Washington in 1907 (Renney 1959). According to Maddox (1977) this weed covered 756,000 acres in Washington, 750,000 acres in Oregon and 73,000 acres in Idaho. The main economic loss from diffuse knapweed is caused by the elimination of useful forage from the rangelands. As forage, this weed has little nutritive value and high fiber content and high levels of consumption can cause toxic symptoms to grazing livestock, especially horses (Maddox 1977). Although herbicide treatments can control this weed the cost is often prohibitive. The weed may be effectively controlled by 1 1/4 pound Tordon 22 k per acre which costs about $15.00. Since knapweed infestations occur extensively on land of low economic value and yield, this is an added impediment to the (chemical) control of knapweeds on western rangelands (Maddox 1977). Because of the economics of knapweed control, biological control of this weed has been investigated and to date

three insects have been found to be host specific. In 1970, a trypetid fly Urophora affinis Frfld., which attacks the seed of diffuse and spotted knapweed, was released in Canada and later (1973) in western United States. In spring of 1976, a root boring beetle, Sphenoptera Jugoslavica Obemb. was released in British Columbia (Canada) and in 1981 in the United States.

DISTRIBUTION AND HOST PLANTS

A literature search conducted did not provide any information on the distribution of Pterolonche inspersa or its host plants. The information in this report on hosts and distribution are based on early findings by Dr. Zwölfer CIBC, USDA field investigations, and personal comunications kindly furnished by Dr. Gozmani (Museum-Allattara Budapest).

Pterolonche inspersa was first described by Staudinger in 1859 and according to Gozmani, the family Pterolonchidae is comprised of a single small Pterolonche genus with a few species. While there is some taxonomic confusion at the family level, there seems to be no problem of determination at the species level. In correspondence with Gozmani he indicated that P. inspersa has been collected in Spain, France, Soviet Union, Hungary, Greece, Turkey, Bulgaria, Romania and Italy. Precise locations for these collections were not given and the reference for Italy is probably a very old record from Centaurea diffusa or collections from C. maculosa, both of which were recorded from northern Italy (now Yugoslavia). Recent investigations conducted in southern Italy by the Authors revealed the presence of Pterolonche albescens on Centaurea alba. This determination was made by Dr. Gozmani. According to Dr. Zwölfer's CIBC reports, P. inspersa was collected from root of C. diffusa, C. maculosa, and C. paniculata in northern Greece in a survey made between 1961 and 1971 (Schroeder 1977).

Field investigations conducted by USDA workers in Northern Greece on various thistles species (Onopordum Cirsium, Carduus, Sonchus and Centaurea) growing sympatrically with C. diffusa revealed that P. inspersa larvae were absent from these genera and found only on roots of C. diffusa.

LIFE HISTORY

Laboratory Biology

MATERIALS AND METHODS

Living insects needed for conducting host specificity and biological studies were collected in northern Greece during June-July from 1981-1984. Infested roots of C. diffusa containing last instar larvae and pupae were dug up in Greece and sent to the Rome laboratory where the infested roots were transplanted in pots (diam 35 cm) containing sand and garden soil 1:3. For rearing-out the insects, each pot was covered by a transparent plastic cylinder cage (diam 30 cm; height 50 cm) with four holes (10 cm diameter) on the sides covered with organdy cloth to permit air circulation. Each cage was capped with organdy cloth held in place by a large rubber band. The rearing was carried out in greenhouse where the temperature ranged between 15-30°C; RH 40 - 80% (outside the cylinder) and the photoperiod was ca. 16 hours. Roots were lightly watered when the soil became dry. The emerging adults were collected daily from the rearing cages, sexed kept outdoors under natural climatic conditions (July-August). To furnish food to the P. inspersa adults, a honey/water solution (5%:95%) was sprayed on the walls of each cage twice a day at 0800 and 1500.

In order to collect eggs for host specificity tests, potted rosettes of C. diffusa were exposed to the ovipositing females ca. 10-12 days and then removed and checked for eggs under a stereomicroscope. During oviposition period, the outside laboratory temperature ranged between 10-35°C, RH 40/85

and photoperiod ca. 16 hours. All eggs found were removed with a fine camel hair brush and transferred into hatching containers made from 50cc plastic cups with a 5mm layer of plaster of Paris on the bottom and capped by a plastic lid with a central hole, plugged with cotton to prevent condensation. After being placed in the oviposition cups, eggs were mantained in the laboratory quarantine room until eclosion.

RESULTS

Under laboratory conditions, emergence took place between the second half of July and the end of August, with peak emergence in mid-August. Males and females emerged simultaneously throughout the emergence period. Emergence under field conditions could not be investigated with precision but it seems to follow the same pattern. Moths emerged from 83% of the roots kept in the sand-soil, with a sex ratio of 1o; 1.5^{o}. In the laboratory females mated within 24 hours of emergence and the preoviposition period lasted 2.6 $\pm$ 0.8 days. The oviposition period lasted 7.4 $\pm$ 2.2 days and the average number of eggs per female was 142. Eggs were oviposited singly or in small groups (up to six) usually on the host-plant leaves, with a slight preference for the lower leaf surface. The distribution of eggs in screen cages (90x90x90 cm) gave the following results: about 50% of eggs were found on the host plant and the remaining eggs were found on the upper part of the cages on the screen cage covering making the results of the oviposition tests conducted in a cage very questionable.

The eggs of P. inspersa are oval, slightly depressed in the middle, with a tough black chorion which has a reticular sculptured surface, with light ridges. The mean egg measurement (n=50) 0.039 $\pm$ 001mm long and 0.025 $\pm$ 001mm wide. The mean hatching period (n=100) lasted 12 $\pm$ 4.7 days. Under laboratory conditions the average longevity of males (n=10) was 10.7 $\pm$ 1.4 days, and 15.8 $\pm$ 2.6 days for females (n=10).

LARVAL POPULATION IN THE FIELD

MATERIALS AND METHODS

The abundance and frequency distribution of P. inspersa larvae were studied at various localities in Greece, where C. diffusa is very common in open areas and disturbed sites along roads usually on sandy and/or gravelly soil. In order to get data on the rate of larval infestation and make observations on the biology, field dissections of various stages of C. diffusa were made in northern Greece during June-July of 1980, 1981, 1982 and 1983.

At each locality (n=10), a random sample of 25-50 plants of C. diffusa was dissected and information was recorded.

RESULTS

During these past years the rate of infestation of diffuse knapweed by P. inspersa in northern Greece remained almost constant, i.e. 20 to 30% infestation at most localities, with a maximum of 75% at the smallest collection site with the lowest plant density.

Field observations showed the rate of attack is apparently correlated with plant density. Small isolated groups of C. diffusa were generally heavily attacked by P. inspersa while in fields with a high plant density the rate of infestation was under 10%.

Dissection of 500 infested roots of different diameters (min 2mm max 52mm) revealed that P. inspersa larvae can be found mining the woody part of the root, or feeding under the epidermis in which case they make a silken tunnel which may be up to 3-5 cm long and 2-2.5 cm wide. The larval feeding site on the root (internal or external), most probably depends on where the egg was laid on the plant. For example, larvae from eggs put near the center of the plant probably mine the central, woody portion of the root while larvae from eggs placed on the peripheral portion of the plant feed on the outside part of the root.

Laboratory and field observations have shown P. inspersa overwinters as a medium size larva (probably 3rd instar). In the spring when the temperature in the field increases larvae start to feed and continue until they become mature. As the larvae feed they spin a peculiar silken tube, lining the gallery they have made, or make a silk tent over the area where they have fed. This tube probably offers both protection during larval and pupal stage, and an easy exit from the root for the emerging adults.

Laboratory and field dissections of infested roots made between September 1981 and July 1982 revealed that P. inspersa is univoltine and that it can co-exist with Sphenoptera jugoslavica (Coleop: Buprestidae) with no deleterious effect on the development of either species.

Mortality factors:

Interspecific competition and larval parasitism were the two mortality factors observed during the study. In laboratory, a large number of first instar larvae were found dead both outside and inside C. diffusa roots. The multiple colonization of the limited internal root space (not more than 2 larvae can develop in a root of C. diffusa) very often results in cannibalism which drastically reduces larval population in a heavily infested plant. The incidence of parasitism was not great. Out of 500 silken tubes (collected in various localities in Greece) and dissected, only 15 contained larvae which were parasitized by Bithia sp. (Diptera: Tachinidae). No observations were made on egg parasites and egg predation was not investigated.

Effect of P. inspersa on host plants. P. inspersa larvae cause the following damage to the host plant: (1) Loss of root wood texture; (2) decrease of plant height; and (3) reduction in size of aerial plant part.

Generally, roots of C. diffusa are small by nature, so many are seriously damaged by one or more larvae feeding in each plant. An attacked root becomes swollen and spongy and looses its normal woody texture, thus reducing the storage capacity and becoming susceptible to invasion by soil-borne organisms. In addition, secondary roots become fragile and break easily, allowing the plant to be removed from the ground with ease. It was also observed that in addition to reducing the height of the plant larval damage in the root gives the plant aerial part a color and shape different from a healthy plant. Plants which survived infestation by P. inspersa produced flowering shoots which were clearly smaller and shorter than shoots on healthy plants.

MATERIALS AND METHODS

Test plants

Plants were chosen for larval survival tests according to three requisites: (1) Plants systematically close to the host plant (under Campanulales, the families Caryophillaceae, Papaveraceae Leguminosae, Euphorbiaceae, Violaceae and Compositae were included); (2) Plants upon which, (Schroeder 1977), Pterolonche inspersa larvae had been collected, including economically important relatives of these plants and various cultivated plant species chosen according to their availability; (3) Biotypes of American Centaurea diffusa and Cirsium spp. Some European species were also included. Seeds were obtained from various commercial dealers and botanical gardens, and the U.S. biotypes of Centaurea and endangered Cirsium spp, were furnished by BCWL, Albany, California. Whenever possible test plants were allowed to flower, voucher herbarium specimens were made to be put in the plant

collection of the BCWL Rome. Most plants were grown in greenhouses but some were grown in the laboratory garden. A total of 62 plant species in six families were tested in the larval survival test, and five species including two commercial flowers and three Centaurea spp. were used in the oviposition choice test.

Oviposition choice tests: To determine the oviposition preference, 7 ♀♀ and 7 ♂♂ adult moths were confined for 10 days in each of 4 cages with one of the following potted plants: Centaurea diffusa (Control); C. diffusa USA; C. soltitialis; C. cyanus; Zinnia elegans. The screen cages used in the tests were 90x90x90 cm and 90x90x160 cm, two of each size were used (2 replicates) for each plant species tested. The tests were conducted in August 1981 in the laboratory garden under natural conditions. The outside temperature ranged between 18-37°C, RH 40-90%, and day length ca. 16 hours. Results are presented in table 1, and 1A.

Larval Starvation test. To determine the host plants that would support P. inspersa development in the laboratory, neonate larvae were transferred from the hatching cups to the center of the test plants. Five replicates were used for each plant species, and each plant was infested with 5 larvae. The experiment was kept in a greenhouse (1981-1982), during the months of August and September. The plants were dissected in October under a stereomicroscope. Results of these dissection are presented in tables 2-3-4.

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Table 1. Oviposition choice test of Pterolonche inspersa (Lep.: Pterolonchidae) in out door cage (90x90x90cm) (Seven pairs were caged on each replicate) 1981.

Replicate No	1	2	Total
Plants tested	No. Eggs laid		
Centaurea diffusa (control)	207	195	402
Centaurea diffusa 1/	161	199	360
Centaurea soltitialis	12	0	12
Centaurea cyanus	1	0	1
Zinnia elegans	0	0	0

1/ American biotype of Diffuse Knapweed (Montana).

Note: In Both replicates a total of 652 eggs were laid on the cage walls (1st Rep 302 eggs - 2nd Rep 350).

Table 1A. Oviposition choice test, of Pterolonche inspersa (Lep. Pterolonchidae) in out dooor cage (90x90x160 cm). (Seven pairs of adults were caged on each replicate) 1981.

Replicate No	1	2	Total
Plants tested	No. Eggs laid		
Centaurea diffusa (control)	136	111	247
Centaurea diffusa[1]	129	109	238
Centaurea solstitialis	0	0	0
Centaurea cyanus	0	0	0
Zinnia elegans	0	0	0

1/ American biotype of Diffuse Knapweed, (Montana).

Note: In both replicates a total of 797 egge were laid on the cage walls (1st Rep 404 eggs - 2nd Rep 393).

Table 2. First instar larval survival test, of Pterolonche inspersa (Lep: Pterolonchidae). (Five 1st instar larvae placed on each plant replicate) 1981.

Replicate No	1	2	3	4	5	Total	% larvae
Plants tested	No.	Larvae	alive	at	dissection		surviving
Fam. COMPOSITAE							
Tribe. CARDUEAE							
Centaure diffusa (Control)	2	2	2	2	2	10	40
Centaurea diffusa 1/	2	2	1	2	2	9	36
Centaurea diffusa 1/	2	2	2	1	2	9	36
Centaurea solstitialis	0	0	0	0	0	0	0
Centaurea cyanus	0	0	0	0	0	0	0
Cynara scolymus 2/	0	0	0	0	0	0	0
Carthamus tinctorius 2/	0	0	0	0	0	0	0
Tribe. Heliantheae							
Helianthus annuus	0	0	0	0	0	0	0
Helianthus tuberosus	0	0	0	0	0	0	0
Tribe. Astereae							
Aster chinensis	0	0	0	0	0	0	0
Tribe. Anthemideae							
Achillea millefolium	0	0	0	0	0	0	0
Tenacetum vulgare	0	0	0	0	0	0	0
Tribe. Cichorieae							
Cichorium intybus 2/	0	0	0	0	0	0	0

1/ American biotypes of Diffuse Knapweed, (Montana Whashington).
2/ Seeds furnished by U.S. Commercial dealers.
Note: 2nd and 3rd instar larvae were recovered from the experiment.

Table 3. First instar larval survival test of Pterolonche inspersa (lep,: Pterolonchidae) (Five 1st instar larvae placed on each plant replicate) 1982

Replicate No.	1	2	3	4	5	Total	% larvae
Plants tested	No.	Larvae	alive	at	dissection		surviving
Fam. Caryophyllaceae							
Tribe. Lychnideae							
Silene vulgaris	0	0	0	0	0	0	0
Silene armeria[1/]	-	-	-	-	-	-	-
Silene nutans	0	0	0	0	0	0	0
Fam. Papaveraceae							
Tribe. Papaverae							
Papaver somniferum[1/]	-	-	-	-	-	-	-
Fam. Leguminosae							
Tribe. Trifolieae							
Medicago sativa							
Fam. Euphorbieae							
Euphorbia lathyris	0	0	0	0	0	0	0
Fam. Violaceae							
Tribe. Violeae							
Viola - ciocca[1/]	-	-	-	-	-	-	0
Fam. Compositae							
Tribe. Condueae							
Centaurea diffusa (control)	2	4	3	3	4	16	64
Centaurea diffusa[2/]	2	5	3	2	3	15	60
Centaurea diffusa[2]	2	3	2	2	3	12	48
Centaurea axillaris	0	0	0	0	0	0	0
Centaurea calcitrapa	0	0	0	0	0	0	0
Centaurea cineraria	1	0	0	0	0	1	4
Centaurea cristata	0	0	0	0	0	0	0
Centaurea crithimifolia	0	0	0	0	0	0	0
Centaurea friderici	1	0	1	0	0	2	8
Centaurea jacea	0	0	0	0	0	0	0
Centaurea rhenana	0	0	0	0	0	0	0
Centaurea scabiosa	0	0	0	0	0	0	0
Centaurea splendens	0	0	0	0	0	0	0
Cirsium discolor[3/]	0	0	0	0	0	0	0
Cirsium andrewsii[3/]	0	0	0	0	0	0	0
Cirsium occidentale[3/]	0	0	0	0	0	0	0
Cirsium Lanceolatum	0	0	0	0	0	0	0
Cirsium palustre	0	0	0	0	0	0	0

1/ Plant spp not sincronized with P. inspersa emergence.
2/ American biotypes of Diffuse Knapweed (Oregon, Idaho).
3/ Endangered U.S. plant.
NOTE: 2nd and 3rd instar larvae were recovered from the experiment.

Table 4. First instar larval survival test of Pterolonche inspersa (Lep: Pterolonchidae). (Five 1st instar larvae placed on each replicate) 1983

Replicate No	1	2	3	4	5	Total	%larvae
	No.	Larvae	alive	at	dissection		
surviving							
Centaurea diffusa (control)	2	1	2	2	1	8	32
Centaurea cineraria	0	0	0	0	0	0	0
Centaurea friderici	0	0	0	0	0	0	0
Centaurea corsiana	0	0	0	0	0	0	0
Centaurea solstitialis var. schowii	0	0	0	0	0	0	0
Cirsium undulatum 1/	0	0	0	0	0	0	0

1/ Endangered U.S. plant.
Note: 2nd and 3rd instar larvae were recovered from the experiment.

Centaurea diffusa Lam (Diffuse Knapweed)

P. H. Dunn, G. Campobasso, and C. Marangoni

INTRODUCTION

During 1985 the project followed the plan developed in 1984 fairly closely. Various insects for use in the tests at Rome or for shipment to the United States were made. New areas and well-known places in Greece were surveyed for the presence of B. fausti, a weevil, that develops inside the diffuse knapweed seedhead.

Sphenoptera jugoslavica Obemb.: In order to provide material for release in the United States a root collection containing mature stages of the Buprestid S. jugoslavica Obemb. was made in Greece during May. About 1,000 infested roots of diffuse knapweed were sent to Albany, California where they were kept in quarantine until adults emerged. Future collections of this beetle will require more time and money because the places where these were known populations in Northen Greece are almost exhausted.

Bangasternus fausti Reitter belongs to the family Curculionidae, subfamily Cleoninae, and tribe Lixini. In a revision of the genus made by Colonnelli, B fausti is present in the following countries: Italy, Romania, Bulgaria, Macedonia, Greece, Turkey, Armenia, and Iran. Centaurea diffusa; C. calcitrapa and C. solstitialis are the only two host plants discovered so far.

In 1981 when work started with this weevil, it was known as B. provincialis Fairmaire, but as a result of a grant from this laboratory for the revision of the genus Bangasternus, it was found that the correct name for this weevil is B. fausti Reitter (Personal Communication Enzo Colonnelli).

The correct determination was necessary because it clarified information (host plants, geodistribution etc.) found in the literature using the first determination, B provincialis.

Survey and Collection trip

In mid-May a trip was taken to Greece to investigate insects associated with the diffuse knapweed. Emphasis was placed on finding populations of B. fausti. Field observations in two areas (Thermi, and Panorama) near Thessaloniki showed this weevil was common on diffuse knapweed, and occasionaly found on yellow starthistle. Twenty-three plant species occurring naturally with diffuse knapweed, were carefully checked in order to define the host range of B. fausti adults. Samples ranging between 38-60 plants of the various sympatric species were checked and the number of B. fausti found on them were recorded (see table 1).

Also, notes were taken on the abundance and frequency of B. fausti and the weevil Larinus minutus (presently under study by C.I.B.C.) on diffuse knapweed at Thermi and Panorama. A sample of 100 plants of diffuse knapweed taken at random were checked per each location and insects found were recorded. Results are summarized on table 2.

To provide material for the 1985 Rome laboratory experiments 700 adults of B. fausti were collected in Panorama near Thessaloniki. At the Thermi laboratory, these weevils were separated by sex, packed and sent to the Rome laboratory at the end of May.

Host specificity tests and Biological notes.

Two series of tests were conducted on B. fausti at Rome:

(1) Oviposition-no choice test; and

(2) Larval-survival test

MATERIAL AND METHODS

Fifteen plant species in the family Compositae (table 3) were included in the no-choice oviposition test. Selection of test plants was based on those used in the host specificity test for B. orientalis. Emphasis was placed on the close relatives of known hosts in the genus Centaurea, and economic crop plants. Prior to the test B. fausti adults were caged on the host plant (C. diffusa) for three days to feed. Subsequently the weevils were sexed and divided into groups of 2 ♂ and 2♀ and each group was confined to a test plant in a pot covered by a transparent plastic cylinder (diam 20; height 70 cm) with four holes (10 cm diameter) covered with organdy on the side of each cylinder. At the top, each tube was capped with organdy cloth held in place by a large rubber band.

Test plants were checked every three days during which observations on the adult feeding damage was recorded. The test was set up in the quarantine room with fluctuating temperature and humidity (Min. 15°-Max. 30°C; RH Min. 30%-Max. 70%), with a photoperiod of ca. 16 hours. The experiment lasted until the insects died. Eggs found on test plants were recorded and left undisturbed to see if resulting larvae were able to reach the adult stage. A summary of the number of replications, average egg production, and adults emerged on test plants is shown in table 4.

Larval Survival test: The same plant species used in the preceding experiment were used in this test. Two buds on each of the 15 test plant replicates were infested with 2 fertile eggs of B fausti (total of 30 eggs per test plant). Also this experiment was carried out in the quarantine room under the same climatic conditions as the above described test. A camel hair brush was used for placing the fertile eggs of B fausti between the bracts of the immature buds of the test plants. All buds used in the test were marked, in order to follow the egg's hatching and larval development. The test lasted

30±2 days, the time required for B. fausti to complete its larval development. Results are summarized in table 5.

Laboratory Colony

Since eggs of B. fausti and diffuse knapweed plants were available, a laboratory colony was started in June in order to have unfed adults of B. fausti to use in an oogenesis test next season. About three hundred buds of C. diffusa were each infested with two fertile eggs and left undisturbed until larval development was completed. Larval development was followed by dissecting a random sample of 5 infested buds each week. At the end of July all diffuse knapweed infested buds were removed (from the mother plants) and placed in cardboard rearing cups (200cc) containing peat moss and kept in a controlled temperature room with fluctuating temperatures, 15° to 30°C, RH 30%-70% and ca. 16-hour photophase.

Larval effect on seed production

During larval development, B. fausti consumes a certain amount of seeds contained in the capitulum of C. diffusa, and damages others reducing their ability to germinate. A laboratory trial was made at the end of June to quantify larval seed consumption and damage caused to the remaining seeds. A random sample of 20 each infested and uninfested seedheads were dissected, and seeds in both categories were counted and recorded. In addition a germination test was conducted with seeds collected from both infested and uninfested seedheads in order to ascertain the percent of seed germinability. Each seed was individually planted in peat moss seed starter (Jiffy-7) and mantained in a greenhouse at ambient temperature for about 60 days. The seed sample taken for this trial was heterogeneous, because only 5 seeds apparently good seeds were found in infested seedheads (20 seeds were taken from uninfested seedheads and 5 from the infested seedheads).

Mortality factors noted during larval development

Observations on the high mortality rate occurring during larval development of B. fausti were carried out in the Rome laboratory during June-July. Seedheads of diffuse knapweed containing fertile eggs of B. fausti were checked and dissected until the new generation of adults emerged. Once a week a random sample of 10 infested seed heads of diffuse knapweed were dissected and material found was recorded.

RESULTS AND DISCUSSION

Oviposition no choice test: The oviposition test revealed that Centaurea diffusa and C. alba were the preferred species for oviposition. Apparently, B. fausti oviposition was influenced by the stage of the floral bud (fig.1). Bud stages 1 and 2 were preferred to hardened tissues of older buds. No oviposition occurred in the other plant species included in the test. Although, the results obtained from this test are satisfactory they are different from what we expected, because not a single egg was laid on Centaurea solstitialis and C. calcitrapa, two known hosts of B. fausti in Greece. We have not discarded the hypothesis that there are different specialized biological host races in this species and perhaps the non-acceptance of C. soltitialis and C. calcitrapa can be in part due to the existence of these host races. Adult feeding damage occurred only on the control and three species of Centaurea: C. alba, C. solstitialis and C. cyanus, none of which are plants of economic importance. On the weed Carthamus lanatus it was noticed that many adults were entrapped in the long hair of the seedhead. Once trapped the weevils were unable to free themselves. The mechanical barrier created by the dense pubescence of the plant protects the capitula from oviposition by small weevils by entrapping them.

Larval survival test:

Of the 13 plant species tested larvae of B. fausti completed their entire development, pupated, and produced adults only on C. diffusa. Larvae fed little or not at all and none developed to the next instar on any of the other test plants. The three composites of agricultural importance, (Cynara scolymus, Carthamus tinctorius and Lactuca sativa) included in the trial did not support larval development. Only first instar larvae were found dead outside of the infested seedheads.

These results clearly indicate the narrow range of host plants capable of supporting development of the newly hatched larvae to the adult stage. Although some Centaurea spp. in the oviposition test provided sufficient attraction for the female to initiate oviposition, not a single plant, with the exception of C. diffusa, demonstrated an attraction for the first instar larvae of B. fausti in the larvae survival test.

Larval effect on seed production

Diffuse Knapweed seed heads infested by B. fausti generally dry up before the noninfested heads. Infested heads may be easily recognized because they are dessicated (color turns from green to pale brown) and sligthly opened, while uninfested heads with maturing achenes are closed and have a different color. Seeds from infested heads are shrivelled and dessicated. Germination tests showed that none of the seeds collected from infested heads germinated, while seeds collected from uninfested heads have shown an high degree of germinability, about 90% in 6-7 days. The results obtained from this preliminary trial may indicate that larval feeding of B. fausti also damages the unattacked seeds in the infested seedheads.

Mortality factors observed during larval development

From about one thousand eggs oviposited on the various seedhead stages and other plant part by B. fausti adults, only one hundred twenty adults (12%) emerged. Apparently, three factors seemed to be the causes of pre-adult mortality: (1) Initial competition between 1st instar larvae in colonizing the internal stem space of the host plant; (2) Cannibalism between larvae in the seedhead; (3) Seedhead size could be the third limiting factor in adult production, i.e. eggs are laid on a seedhead that never gets large enough to support a larva to maturity.

In artificial conditions, when all seedheads of the host plant are already occupied by eggs, the adults of B. fausti oviposit on stems. Usually, those eggs do not have much probability of reaching the seedhead alive. Factors, like long distance (4-5 cm, from egg deposition site to seedhead) and the limited stem space available for larval feeding drastically reduces larval population. It was observed that stems already colonized, were not attacked by other larvae. We found that not more than one larva, can reach the seedhead through the stem. Cannibalism between medium size larva of B. fausti was observed in seedheads containing more than onr larva.

Usually the surviving larva occupies the center of the receptacle while the dead ones were found in the internal bracts close to the receptacle. Seedhead size had a very important role in larval development. The adult choice of a wrong egg oviposition site may cause loss of a developping larva. Vigorous plants of diffuse knapweed normally produces various size of seedheads, some of which are not able to provide sufficient food for completing larval development. Our observations showed that only seedheads with a minimum diameter of 4.5 mm contained enough food to allow a larva to

reach the imago. Dissection of smaller (2.3 mm diameter) seedheads clearly showed that larvae of B. fausti were able to complete just the first phase of first instar stage because of limited food. These results may be helpful if future laboratory rearings of B. fausti are attempted.

Laboratory colony

About one hundred-forthy adults of B. fausti were obtained from three hundred seed heads infested in June. Some of the adults emerged from the seedhead at the beginning of October and tried to find a refuge in the peat moss put in the emerging containers. Observations made during winter on the adult behavior did not show much. Most of the time, the adults were found motionless in the peat moss. Most of the adults will be used for an oogenesis test in the next season.

CONCLUSION

The following are the justifications for continuing the screening of the seed feeder weevil B. fausti Reitter.

1. A literature search did not give any record of the weevil damaging cultivated plants.
2. Field investigations conducted in Greece in different plant genera showed that adult host range was restricted to the genus Centaurea.
3. Adult populations in the field were abundant, thus massive collections can be made in a short time.
4. The no-choice oviposition test in laboratory proved that only plants in the genus Centaurea were accepted by B. fausti adults. Endangered U.S. plant species included in the experiment were not damaged nor did the females lay eggs on them.

5. Larval survival test clearly showed that larvae of B. fausti were able to complete their development only on diffuse knapweed. Larvae used in transfers to economic, endangered, and native plant species died before moulting the 2nd instar without causing damage to the test plants.

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Work Plan for 1986

Project: Diffuse Knapweed

The following plants will be tested with *Bangasternus fausti*:

1. *Centaurea diffusa* (control) Greece
2. *Centaurea diffusa* U.S.A.
3. *Centaurea cineraria*
4. *Centaurea jacea*
5. *Centaurea scabiosa*
6. *Centaurea splendens*
7. *Cirsium discolor* (U.S.A.)
8. *Cirsium andrewsii* (U.S.A.)
9. *Cirsium lanceolatum*
10. *Cirsium occidentale*
11. *Cirsium palustre*
12. *Carduus pycnocephalus*
13. *Senecio cineraria*
14. *Xeranthemum annuus*
15. *Lactuca sativa*
16. *Cichorium intybus*
17. *Onopordum acanthium*
18. *Papaver somniferum*
19. *Aster chinensis*
20. *Cynara scolymus* (U.S.A. varieties)
21. *Carthamus tinctorius* (U.S.A. varieties)

Collection trip

About twenty days will be spent in Greece to collect Bangasternus fausti, Sphenoptera jugoslavica, and Pterolonche inspersa. About 500^{oo} and 500oo will be collected at the end of May for oviposition tests and larval survival tests in Rome. Thistles growing sympatrically with C. diffusa will be checked on possible hosts of B. fausti. Massive collections of S. jugoslavica and P. inspersa will be made in northern Greece and shipped to Albany, California for release in the United States.

Table 1. Plant species in Greece associated with diffuse Knapweed which were checked for the presence of Bangasternus fausti and Larinus minutus.

Plant species	No. Plants checked	No. insects present	
		B. fausti	L. minutus
Centaurea diffusa (control)	50	70	54
" soltitialis	60	10	0
" salonitana	50	0	0
" macedonica	52	0	0
" maculosa*	50	0	0
" calcitrapa*	53	0	0
Carduus nutans	52	0	0
" pycnocephalus	60	0	0
" candicans	45	0	0
Cirsium lanceolatum	50	0	0
" arvense	50	0	0
Carthamus lanatus	52	0	0
" dentatus	55	0	0
Onopordum acanthium	60	0	0
" illyricum	51	0	0
Sonchus oleraceus	58	0	0
Scolymus hispanicus	57	0	0
Carlina corymbosa	51	0	0
Silybum marianum	48	0	0
Cardopatum sp	52	0	0
Echinops microcephalus	52	0	0
Cnicus benedictus	38	0	0
Tyrimnus sp.	50	0	0

* = No adults of B. fausti were found during our observation, but according to Dr. R. Sobhian's Laboratory in Thessaloniki, Greece, these two plants are hosts of B. fausti (personal comunication).

Table 2. Infestation level of Bangasternus fausti Reitter and Larinus minutus on Diffuse Knapweed, Centaurea diffusa Lam at two localities near Thessaloniki (Greece). 1985.

Location	No Plants Examined	No Plants with adults present		No Plants containing both species	Adults/Plant	
		B. fausti	L. minutus		B. fausti x̄±SD	L. minutus x̄±SD
Thermi	100	24	36	12	0.8±2.0	1.2±1.9
Panorama	100	40	23	22	2.8±4.8	0.7±1.8

Table 3. Plant species used in the host specificity test for B. fausti Reitter.

No	Family	Species	Seed Source
1	Compositae	Centaurea diffusa (control)	Greece
2	"	Centaurea alba	Italy
3	"	Centaurea cyanus	"
4	"	Centaurea calcitrapa	"
5	"	Centaurea solstitialis	"
6	"	Centaurea americana	U.S.A.
7	"	Cynara scolymus	"
8	"	Helianthus annuus	"
9	"	Carthamus tinctorius	"
10	"	Carthamus lanatus	Italy
11	"	Zinnia elegans	"
12	"	Cirsium arvense	"
13	"	Cnicus benedictus	"
14	"	Chrisanthemum leucanthemum	"
15	"	Cirsium douglasii	U.S.A.

Oviposition no choice test 1985.

Table 4. Summary of Bangasternus fausti Reitter

Test plants	Total No. of Replicates	Total No. of insects in replicates		No. seed heads exposed/rep		No. seedheads infested/rep		% of seedheads infested/rep		No. eggs oviposited replicates		No. of adult Emerged/rep	
		oo	oo	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Centaurea diffusa (control)	10	20	20	156.7	33.5	102	12.5	71.9	22.5	213	89.6	13.8	4.1
Centaurea alba	10	20	20	39.6	11.1	19	5.3	43.3	30.3	58.8	52.2	-	-
Centaurea cyanus	10	20	20	71.5	13.8	-	-	-	-	-	-	-	-
Centaurea calcitrapa	10	20	20	33.4	8.3	-	-	-	-	-	-	-	-
Centaurea solstitialis (USA)	5	10	10	15.8	4.3	-	-	-	-	-	-	-	-
Centaurea americana (USA)	1	2	2	-	-	-	-	-	-	-	-	-	-
Cirsium arvense	10	20	20	12.2	2.5	-	-	-	-	-	-	-	-
Cirsium douglasii (USA)	1	2	2	-	-	-	-	-	-	-	-	-	-
Cnicus benedictus	10	20	20	6.4	1.9	-	-	-	-	-	-	-	-
Carthamus lanatus	10	20	20	15.3	2.1	-	-	-	-	-	-	-	-
Carthamus tinctorius (USA)	10	20	20	14.5	2.5	-	-	-	-	-	-	-	-
Cynara scolymus (USA)	6	12	12	2.5	0.7	-	-	-	-	-	-	-	-
Helianthus annuus (USA)	10	20	20	2.8	1.1	-	-	-	-	-	-	-	-
Zinnia elegans	10	20	20	5.8	1.8	-	-	-	-	-	-	-	-
Chrisanthemum leucanthemum	10	20	20	16.9	2.8	-	-	-	-	-	-	-	-

Table 5. First instar larval survival test of B. fausti.

Plant	No. replications	Total eggs used	Total eggs hatched	Feeding	Max development stage reached				
					instars				
					1st	2nd	3rd	Pupa	adult
Centaurea diffusa (control)	15	30	22	Heavy	0	0	8	5	2
Centaurea alba	15	30	27	Ligth	21	0	0	0	0
Centaurea cyanus	15	30	26	Ligth	19	0	0	0	0
Centaurea calcitrapa	13	26	24	None	24	0	0	0	0
Centaurea solstitialis (USA)	15	30	29	None	23	0	0	0	0
Centaurea americana (USA)	1	2	2	None	2	0	0	0	0
Cirsium arvense	15	30	22	None	18	0	0	0	0
Cirsium douglasii (USA)	2	4	4	None	3	0	0	0	0
Cnicus benedictus	15	30	25	None	21	0	0	0	0
Carthamus lanatus	15	30	25	None	23	0	0	0	0
Carthamus tinctorius (USA)	15	30	25	None	23	0	0	0	0
Cynara scolymus (USA)	7	14	14	None	13	0	0	0	0
Chrisanthemum leucanthemum	15	30	22	None	16	0	0	0	0
Zinnia elegans	15	30	22	None	17	0	0	0	0
Helianthus annus (USA)	15	30	27	None	25	0	0	0	0

a/ All 1st instar larvae found on test plants were dead; b/ 3rd instar larvae, pupae, and adults found on the controls were alive and very active.

Table 6. Comparison of seed production of two Diffuse Knapweed seedhead categories (infested and not infested by *B. fausti* larva).

Seedhead Dissected	No. seedheads contained/each unattacked seedhead	No. seedheads contained/each attacked seedhead
1	23	0
2	20	0
3	13	0
4	15	0
5	16	1
6	20	0
7	21	0
8	19	0
9	18	0
10	17	1
11	21	0
12	19	0
13	17	0
14	22	0
15	14	2
16	21	0
17	22	0
18	16	1
19	18	0
20	15	0

Note = A sample of seeds from both categories of seed heads were planted in jiffiseven, but only seeds of unattacked seedhead germinated.

Musk Thistle Carduus macrocephalus

P. H. Dunn, G. Campobasso, M. Stazi

INTRODUCTION

An open field experiment, testing American plants in the genus Cirsium, Carduus, and Cynara, was made with the crown-feeding weevil Ceutorhynchus (Hadroplonthus) trimaculatus (Col: curculionidae) a potential biological control agent of Carduus spp. in the U.S. The experiment was conducted from December 1984 to March 1985 in a fallow field at Castel Porziano (Rome), in a situation that simulated conditions that might occur if Carduus spp. and Cirsium spp. were to grow together.

MATERIALS AND METHODS

Seeds of the control plant Carduus macrocephalus (Italy), C. macrocephalus (USA), C. acanthoides (USA), Cirsium undulatum (USA), C. flodmanii (USA), C. spinosissimum (USA), C. crassicaule (USA), and Cynara scolymus (USA) were planted in the laboratory greenhouse at the end of September 1984. After the formation of 3-4 leaves all plants were transplanted in 22 cm diameter pots and kept outdoors for acclimation. Insects needed for the experiment (n=31♀♀ and 35♂♂) were collected at Castel Porziano 5 km from the field chosen for the test and brought to the laboratory where they were counted, sexed and marked according to sex.

A field of 64 sq.m. (16 x 4 m) in a site where Carduus macrocephalus and the weevil C. (H) trimaculatus were relatively abundant was chosen for this trial. The field was divided into 27 small blocks (1 m x 1 m) each containing naturally growing C. macrocephalus plants which were used as attraction plants. Each block contained one attraction plant, one potted control, and one potted test plant. Plant position in the block was assigned randomly (see Fig.1). The experiment started in December when 1♂ and 1♀ (marked) were

released in each block. Once a week all the plants were inspected and the number, sex and location of any adult insects found was recorded. The test lasted from December 6 to March 8 when all the test plants were taken from the plot and dissected in the laboratory under a stereomicroscope. The number of larvae and the plants in which they were found were recorded (see table 1).

RESULTS

At each examination, both marked and unmarked (natural population) weevils were found in the plot. The Control plants (both attraction and potted plants) were the ones most frequented by the weevils. At least 2 adults were found on controls at each inspection. Our findings showed that American Cirsium were not visited frequently by adults. In only on one occasion 2♀♀ were found on C. flodmanii; 2♀♀ on C. spinosissimum and 1♂ on C. undulatum. No adults were found on Cynara-scolymus. However, despite the infrequent visits, the results of the plant dissection were not satisfactory. The same Cirsium spp. on which the adults of C. (H) trimaculatus were found were infested with C. trimaculatus larvae. No damage or larvae were found on Cynara scolymus.

Table 1. Open field Experiment with Ceutorhynchus (Hadroplothus) trimaculatus. Rome, Italy - 1984-1985

Plant No.	Number Larvae Found								
	Carduus macrocephalus Control (Italy)	Carduus macrocephalus Attraction (Italy)	Carduus macrocephalus Test (USA)	Carduus acanthoides Test (USA)	Cirsium undulatum 1/ Test (USA)	Cirsium floodmanii 1/ Test (USA)	Cirsium spinosissimum 1/ Test (USA)	Cirsium crassicaule Test (USA)	Cynara scolymus Test (USA)
1	2	7	12	1	0	6	0	- 2/	0
2	4	6	4	5	0	1	0	-	0
3	5	5	10	7	6	0	5	-	0
4	9	3	12	4	0	0	2	-	0
5	5	7							
6	2	1							
7	4	7							
8	6	5							
9	3	10							
10	6	5							
11	9	12							
12	10	9							
13	3	5							
14	4	4							
15	5	1							
16	11	3							
17	17	3							
18	32	3							
19	6	4							
20	4	8							
21	16	6							
22	14	1							
23	10	4							
24	23	2							
25	3	3							
26	23	0							
27	9	5							
TOTAL	245	129	38	17	6	7	7	0	0
Mean No. Larvae/Plant	9.1	4.7	9.5	4.2	1.5	1.7	1.7	0	0
SD	±6.60	±2.79	±3.28	±2.17	±2.60	±2.49	±2.05	0	0

1/ Adult C. (H) trimaculatus found on plant
2/ Plant died during experiment

Yellow Starthistle, (*Centaurea solstitialis* L.)

Thermi, Greece

R. Sobhian

WEED GARDEN-PLOT STUDY

A field experiment was designed by S. Clement and R. Sobhian to fulfill the following objectives:

1. To investigate the ability of *Chaetorellia hexachaeta australis*, *Eustenopus hirtus*, and the rest of the insect guild associated with Yellow starthistle, *Centaurea solstitialis* L., (YST) seedheads in Greece to locate and attack different strains of YST, *Carthamus tinctorius*, *Cirsium creticum* and *Cynara scolymus* under field experimental conditions.
2. To quantify bud and flowerhead development on YST strains from the U.S. and from Thermi, Greece, so the seasonal activity cycles of promising biocontrol agents could be related to the availability of their preferred oviposition sites.

METHODS

A weed garden-plot, consisting of 6 treatments (YST strains, plant species or cultivars) replicated 6 times (randomized complete block design) was established at the University of Thessaloniki Farm in Thermi, Greece. Plant spacing within the row and the distance between two rows was ca. 2 m. The test plants in this weed garden-plot were selected on the basis of (a) botanical relationships to YST, (b) economic importance, (c) native North American plant considerations and (d) the geographical distribution of YST in the Western U.S.

The test plants were:

1. Artichoke (Art.) Cynara scolymus (U.S. cultivar "green globe"); obtained as rootstock from California in 1983. These plants were left in place for this study after they were used in a previous experiment in 1984.
2. Cirsium creticum (C.): Plants were removed from a site near Ag. Prodromos, Greece on October 15, 1984, and transplanted into the plot at Thermi.
3. Safflower (SF.) Carthamus tinctorius (U.S. cultivar Hartman). Seeds were sown at 2 different times in order to have both budding and flowering plants available for an extended period. Seeds were planted in Jiffy-7 (peat pellet jiffy pots)[1/] on March 11, 1985 and kept in a greenhouse until May 10 when they were placed in the plot. The second group of plants were grown from seed planted in the soil on May 10, adjacent to the first group of SF plants.
4. YST seeds from 3 different sources (Thermi, Greece, GR:, Sacramento, California, CA: and Lapwai, Idaho: ID) were planted in pots on September 25, 1984 and kept out-of-doors until they were transplanted into the plot on March 11. In the beginning, three plants were clustered together, but the smaller ones were removed on May 23, 1985 leaving only the largest one for further studies. Fig. 1 shows a layout of the weed garden-plot.

1/ Mfg. by Jiffy Products Ltd., Norway.

The YST plants were not watered but the other plants in the trial were watered as needed. The plot was weeded as necessary in order to permit the plants in the experiment to grow unimpeded. However, not all the weeds were removed at each weeding as it was decided to leave some resting and hiding places for insects.

The test plants were generally in good condition throughout the field test. One of the early SF plants and all three CA-YST plants in one cluster were eaten by moles. Since the YST damage happened before the elimination of the smaller YST plants in the plot a healthy plant was substituted from another part of the plot.

Adults of Eustenopus hirtus collected from June 1 to 12 on YST in an abandoned field behind the cemetery of Thessaloniki. They were brought to a laboratory and caged with YST bouquets. Since we were not able to sex the adults morphologically, males and females were separated as they mated in the laboratory cage, and mating paris were also field collected. Males and females from both laboratory and field collections were kept separate on YST bouquets in the laboratory. The males were labeled with one dot of pink nail polish on their right elytra, and females were labeled with a dot on their left elytra.

The first group of labeled beetles was released in the plot on June 9 (one labeled pair per replicate). A second release of labeled insects was made on June 12, making a total of 144 adults, (72 oo and 72^{oo}) released in the test plot.

All test plants were checked weekly for the presence of individual males and females, mating pairs, and unlabeled adults, from June 13 through July 18 (6 weeks). Yellow starthistle plants growing within 25 m from the plot were also checked for labeled adults. Two people took part in each 3-4 h census.

Thousands of YST seed heads collected in the field around Thermi, some of which were infested with C. hexachaeta australis, were placed in the plot among the test plants to give emerging flies the opportunity to select host plants for oviposition. In addition, a few thousand flower heads were kept in cages both inside and outside the laboratory, and flies that emerged in these cages were released on the plants in the experimental plot.

Flowerheads from all the plants in the test were collected at weekly intervals, from June 21 to August 16, 1985, spiders were removed from the flowerheads (because they would prey upon emerging insects) and the samples were shipped to Rome where the insects were reared out and identified, and the capitula were dissected.

A random number generator was used to select three plants from each YST strain on May 10 and three branches were labeled on each of the nine plants as "low", "middle", and "high". Starting on May 23 the buds and flowerheads on these branches were counted at weekly intervals and their phenological stage according to Maddox (1981) was recorded. The last counts were taken on August 8, when only one CA plant which had only one F2 flowerhead was alive. The rest of the plants had senesced by this date.

RESULTS

Eustenopus hirtus (Coleoptera: Curculionidae)

Table 1 shows the number of individual males and females, mating pairs, and unlabeled Eustenopus counted on each test plant in the plot. The counts are broken down to show the number observed at each census.

The data in Table 1 suggest that U.S. YST were preferred over the local strain. However, a more plausible explanation is that the number of Eustenopus adults found on the three YST strains was related to the number of

Table 1. Number of individual females and males, mating pairs, and unlabeled adults of Eustenopus hirtus recorded on each plant species during 6 observation periods, weed-garden plot, Thermi, Greece 1985.

Test plant	females	males	mating pairs	Unlabeled adults
GR-YST	9	11	5	
	(8,1,0,0,0,0)*	(8,1,2,0,0,0)	(4,1,0,0,0,0)	0
CA-YST	13	11	5	
	(5,2,4,1,1,0)	(5,4,0,1,1,0)	(4,1,0,0,0,0)	0
ID-YST	16	9	6	2
	(12,3,1,0,0,0)	(5,1,3,0,0,0)	(3,3,0,0,0,0)	(0,1,1,0,0,0)
Cirsium creticum	0	0	0	0
Artichoke	0	0	0	0
Safflower	0	0	0	0

* = The six numbers in parenthesis represent counts from each of the 6 censuses.

Fig. 1. Layout of the weed garden-plot, Thermi, Greece, 1985.

ID x	SF	C	Art	GR	C
SF	Art	CA	ID	SF	ID
CA x	C	Art	C	ID x	GR x
Art	GR x	SF	GR	C	Art
GR	ID	ID x	SF	CA x	CA
C	CA	GR x	CA x	Art	SF

GR = Centaurea solstitialis, Greece
CA = Centaurea solstitialis, California
ID = Centaurea solstitialis, Idaho
SF = Carthamus tinctorius var. Hartman
C = Cirsium creticum
Art= Cynara scolymus var. Green Glove

Plants marked with "x" were picked at random for flowerhead count.

available Bu-4 buds, which are the preferred oviposition sites. The maximum number of Bu 4 buds on ID-YST was recorded on June 13 (date of first beetle observation), on June 20 on CA-YST, and on June 6 and 18 on GR-YST. Eustenopus adults were observed for 5 weeks on the California plants. Figure 2 shows the number of beetles and Bu-4 buds recorded on each YST strain at each census.

On June 12, three labeled adults (1 ♀ and 2 ♂♂) were observed 25 m from the plot on US YST plants. On July 18, when adults were no longer found in the plot, one unlabeled female was found on a young YST plant about one meter from the plot.

Chaetorellia hexachaeta australis (Diptera: Tephritidae)

Flies of this tephritid started emerging on April 22, about one month before the YST bud and flowering phase (see Table 3). No Chaetorellia adults were found on YST rosettes or bolting plants in the plot or on nearby plants. In late April 20 C. hexachaeta adults were released on YST plants in the plot but they were not observed in subsequent examinations, apparently having left the area.

On May 24, 300 flowerheads of Centaurea cyanus and 3 large samples of Carduus pycnocephalus capitula (2, 130; 2, 200; and 1,500 heads) were collected near Thermi where C. hexachaeta had been found on YST in 1984. The C. cyanus heads were within 4 meters from the plot.

No flies emerged from C. pycnocephalus heads but 10 C. hexachaeta emerged from 300 C. cyanus heads. The rest of the wild C. cyanus plants near the plot probably supplied the Chaetorellia adults which colonized the YST plants in the plot.

INSECT LIFE HISTORY STUDIES

Chaetorellia hexachaeta australis (Diptera: Tephritidae)

The insect overwinters as mature larvae in seedheads. In order to determine the time of pupation in nature, samples of YST seedheads were collected and dissected from February 21 to May 2. The first pupae were found in the seedheads on March 23; the last larvae were found on April 4. In the sample collected on May 2, only pupal exuviae were found. Table 2 shows the results of the dissections.

Actual adult emergence was monitored by caging samples of YST seedheads at the end of March. There was one sample of several thousand seedheads per cage and each of the cages used was kept under a different condition as shown in Table 3. The cages were checked daily for emergence and the number and sex of the emerging *Chaetorellia* was recorded.

Table 3 shows the dates and the number and sex of adult flies that emerged from the samples. Temperature is most likely a key determinant factor for the emergence of adults. Sunny and relatively warm days characterized the period between April 22 and 26 when all of the adults emerged from the sample in the cage kept under direct sunlight. The ratio of males to females that emerged from the three samples was 1:1, and their emergence was pretty well synchronized (i.e., males did not emerge earlier than females).

Examination of *C. cyanus*, which flowers much earlier than the YST, showed that it is an early host plant for the fly (see also discussion on weed garden-plot). Eight adults emerged on May 30 and June 1 from a sample of 300 *C. cyanus* flower heads collected on May 14 at the University farm at Thermi, demonstrating that the adults emerging in late April and early May can complete a generation in about one month on *C. cyanus*. It is not know if there are flowering plants other than *C. cyanus* that serve as early hosts for the fly.

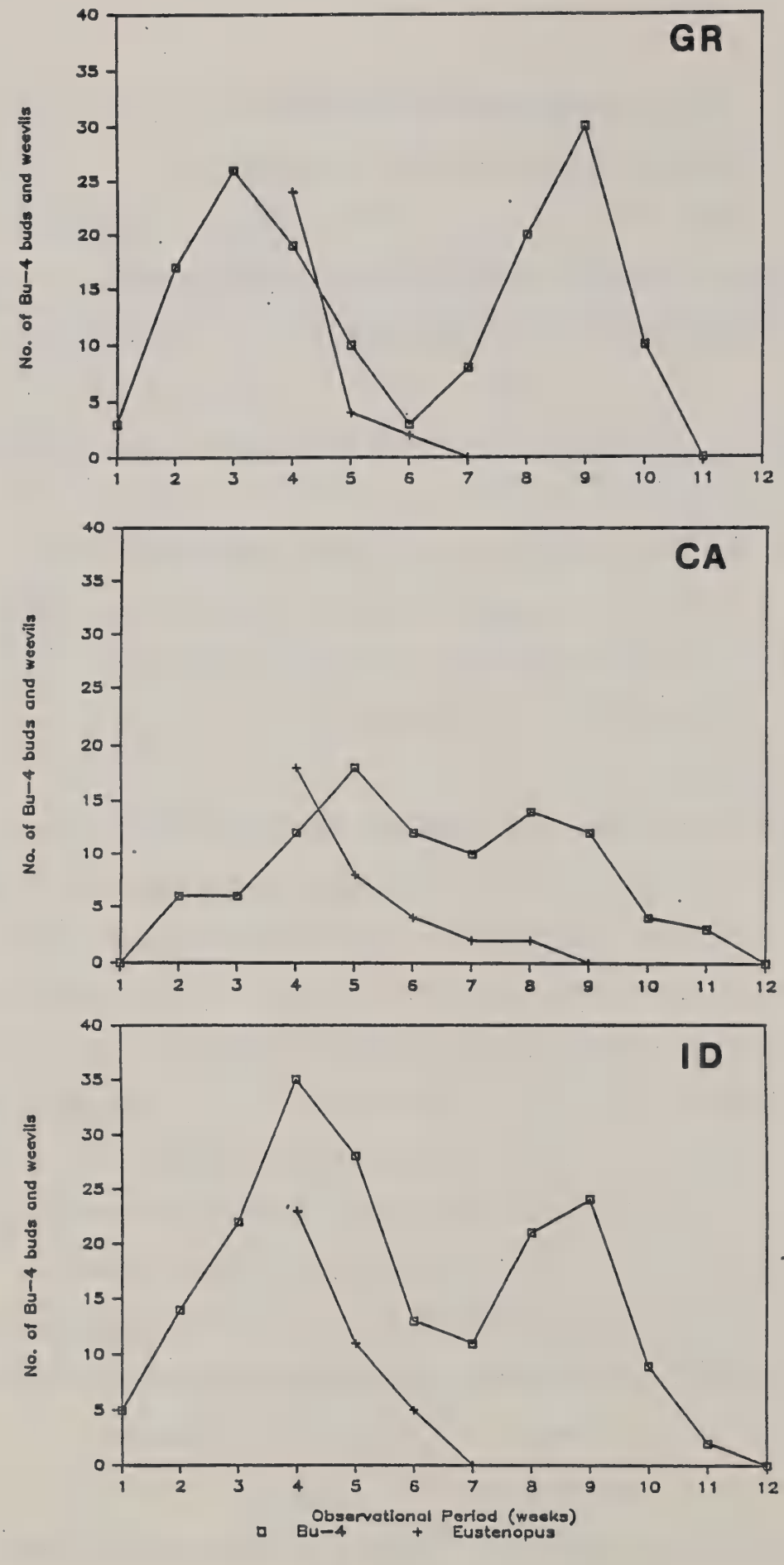


Fig.2 : Number of Eustenopus adults and Bu-4 buds on YST plants during the observational period, Thermi, Greece, 1985.

In Doirani and Geroplatanos (Near Arnea), where C. cyanus is common, the rate of infested YST seedheads was fairly high. We were never able to find natural populations of C. cyanus around Thermi, but C. hexachaeta is present in the area. The percentage of YST seed heads harboring C. hexachaeta australis at Doriani and Geroplatanos was, respectively, 25% (n = 226) and 47% (n = 100). The percentage of infested YST seedheads at Thermi was 0%-5% in nine samples with a minimum 100 seedheads per sample.

In an attempt to determine if C. hexachaeta australis and E. hirtus will attack the same YST capitula (Bu-4 bud) in an area where both insects commonly occur, 295 YST flowerheads in the seed formation stage were collected from Doirani (July 9-16) and dissected under a stereomicroscope. Thirty capitula had both Chaetorellia eggs and Eustenopus oviposition holes. Eustenopus larvae were found in 17 heads, Chaetorellia larvae in 6 heads, and a Chaetorellia pupa and a Eustenopus larva were both in one head. Neither insect survived on six other capitula that served as oviposition sites for both. From these limited observations it is difficult to determine which insect is dominant when they both occur in the same head.

A number of parasites were reared from larvae or pupae of C. hexachaeta recovered during the course of various dissections throughout the season, and they have been submitted to SEL for identification.

Table 2. Data on pupation and emergence of Chaetorellia hexachaeta australis from overwintering larvae, Thermi, Greece 1985.

Date	Feb. 21 to March 19	March 23	March 25	April 4	April 19	May 2
No. heads dissected	17,000	550	600	600	500	500
No. overwintering larvae	210	7	5	0	0	0
No. pupae	0	3	3	8	7	0
No. exuviae	0	0	0	0	0	6

Table 3. Emergence of adult Chaetorellia hexachaeta australis from overwintering larvae in capitula exposed to different conditions, Thermi, Greece, 1985.

Sample Treatment	Date of adult emergence	No. oo	No. oo	End of observation
Cage kept in laboratory	April 8-29	7	8	May 14
Cage kept under direct sunlight	April 22-26	11	8	May 14
Cage kept outdoors in the shade	May 14-24	4	6	May 24
TOTAL		22	22	

Eustenopus hirtus (Coleoptera: Curculionidae)

Beside the weed garden-plot study, some additional data were collected on the bionomics and natural mortality of E. hirtus by collecting five flowerhead samples (see Table 4) between June 13 and September 12 from YST plants near the cemetery of Thessaloniki and dissecting them with the aid of a stereomicroscope.

Table 4 shows the results. The first pupa was found on June 25. In the field, adults seemed to leave the seedheads by August 8, but in the laboratory they showed a tendency to stay in the seedheads through August and into mid September. A large number of parasitized pupae were collected but the adult parasites had not emerged at the time this report was written.

On June 13 two samples of YST seedheads were collected. The results of one are shown in Table 4, and the second sample (n = 136) was kept in the laboratory until the heads were dissected on September 19. In this second sample, 18 of the heads had oviposition holes and we found the following in these: 11 heads were not infested, 6 adults were found (5 living 1 partially eaten and 1 head had a parasitized larva).

The results in Table 4 and from the September 19 dissections suggest that parasites attack fairly mature larvae because the rate of parasitism was higher in the samples collected after June 13 (Table 4). In the future if we wish to obtain large numbers of adults, it might be advisable to collect new YST flowerheads with Eustenopus oviposition holes, prior to June 13, and keep them in the laboratory to avoid parasitism and rear-out the weevils.

Bangasternus orientalis (Coleoptera: curculionidae)

Mating was observed between B. orientalis ♂♂ and B. planifrons ♀♀, and vice versa, in 1984 (see Annual Report 1984) under petri dish conditions, so

Table 4. Infestation and observations on mortality, time of pupation and emergence of Eustenopus hirtus from capitula of Centaurea solstitialis collected near Thessaloniki, Greece, June 13 to September 12, 1985.

Collection Date	No. of Heads Dissected	No. of Heads with Eggs	No. of Living Larvae Present	No. of Parasitized Larvae Present	No. of Pupae Present	No. of Adults Emerged
June 13	100	29	19	0	0	0
June 25	100	30	11	14	1 1/	0
July 2	100	74	10	34	1	0
Aug. 8	390	40	0	25	0	1 2/
Sept. 12	910	234	0	106	0	1 3/

1/ First evidence of pupation

2/ First evidence of adult emergence

3/ 11 empty pupae cells were found, from which adults probably emerged.

the possibility of hybridization between the two species could not be ruled out. Therefore, the study described herein was conducted in an attempt to cross-breed B. planifrons ♀ (=P) and B. orientalis ♂ (=O). The control was B. planifrons males and females (=C). Virgin females of B. orientalis were not available for this study.

METHODS

A sample of Carthamus dentatus seedheads, collected around Thermi on September 6, 1984, was kept in the laboratory until September 28 but no adults emerged by this date. However, dissections of the heads yielded 64 adults, which were allowed to overwinter outdoors in vials plugged with cotton (one adult per vial). When the vials were examined on May 9, 1985 the weevils were not active; some were still resting in their cocoons, but 57 living beetles were removed from the vials and placed in petri dishes (one per dish) and kept supplied with fresh C. dentatus branch tips until they became active on June 12, and it was possible to start the experiment.

On June 12, Bangasternus orientalis oo were labeled with pink nail polish and placed in petri dishes containing B. planifrons adults (sex ratio unknown) and YST branch tips were added to the containers as food for the B. orientalis. When PxO mating was observed the pair was selected for the experiment. By June 6, six such pairs (PxO) had been separated. Two mating B. planifrons pairs were also selected for use as controls (C).

Each pair of beetles (6 replicates of PxO; 2 of C) was caged separately in one liter transparent plastic containers with sufficient aeration. Bouquets of C. dentatus and YST were offered to the PxO pairs, while the C cages contained only C. dentatus bouquets. The bouquets were replaced every second day with fresh plant material.

By July 10, all B. orientalis males, two B. planifrons females in PxO replicates and one of the C females had died. On July 20 a small test was set up with three B. planifrons females from the PxO replicates and one control female. In this test each female was caged without a male on a field grown C. dentatus plant in early June at the University Farm in Thermi. The cages (screened bags) were first placed over the plants before the onset of planifrons oviposition thus assuring the plants to be free of eggs. The plants were examined on September 11, noting the presence of Bangasternus eggs on the plants and immature forms and adults inside heads.

RESULTS

The results are shown in table 5.

A large number of eggs were laid by the single females on the caged C. dentatus plants at the University Farm, but none hatched. The control female laid 28 eggs, 26 of which hatched. Four adults and nine pupae were found in the heads offered to the "Control" female.

The results of the trial presented in table 5 indicate that copulation between B. planifrons and B. orientalis is possible in caged conditions but the resulting eggs are infertile.

Egg parasites: Hundreds of B. orientalis eggs were collected in October 1984 and kept in a petri dish in the laboratory (not heated during the winter). By May 10, 1985, a large number of egg parasites had emerged. The parasites emerge about the same time as the oviposition period started for B. orientalis. Specimens were sent to USDA ARS SEL for identification.

Urophora sirunaseva: (Diptera: Tephritidae)

Table 5. Crossmating experiment, Thermi, Greece, 1985. (see text for explanation).

Replicates and control	No. of seed heads	No. of eggs laid	No. of eggs hatched	No. of eggs not hatched	No. of pupae	No. of adults
P,1[1/]	14	31	0	31	0	0
P,2[1/]	23	39	0	39	0	0
P,3[1/]	12	0[3/]	0	0	0	0
Control[2/] female	17	28	26	2	9	4

1/ These B. planifrons females were first coupled with B. orientalis males.
2/ This B. planifrons female was first coupled with a B. planifrons male.
3/ Insect escaped

Urophora reared from Centaurea solstitialis in Greece, including the specimens reared from U.S. YST, were identified by Dr. R.H. Foote (Research Entomologist, SEL, USDA), as U. sirunaseva in 1981. Additional material was submitted to Prof. H. Zwölfer (University of Bayreuth, Germany) for identification, who confirmed Dr. Foote's identification.

To obtain more Urophora sirunaseva galls from YST of U.S. origin, seeds of U.S. YST were sown in Thermi in fall of 1984. The resulting rosettes were transplanted into a plot at University Farm on March 8, 1985. When buds were available on the plants 30° and numerous o were collected and released on the plants in order to supplement the naturally occurring population. However, the rate of attack was low judging by the 4% infestation rate of a sample of 100 heads from each YST strain. As suggested by D. Maddox, all seed heads (Sacramento 8,500; Woodland 10,000) from the YST plants were collected (August 25-29) and shipped to California for rearing adult flies for release in California in 1986.

GENERAL SURVEYS

In late July, a two and half day survey trip was made with Paul Dunn to sections of northern and central Greece (Kozani, Grevena, Metsovo, Ioannina, Agrinion, Houni). Our main objective was to locate good infestations of Larinus curtus, a potential biocontrol candidate for YST, so large numbers of adults could ultimately be obtained for host specificity tests. Unfortunately, only a few adults were found at three different locations (near Grevena; 40 km. south of Ioannina; and Houni). We were also looking for good infestations of B. orientalis and E. hirtus, but none were found.

A one-day trip was made to Kavala and Drama on August 28 and another to the area around Seres and Nigrita in mid-September. These trips were made to locate good infestations of various seedhead insects for future collections.

A few samples were collected from other areas in northern Greece and two seedhead samples were provided by C. Turner (ARS Laboratory, Albany, California). A total of 14 samples of 100-250 heads per sample were examined and the rate of infestation by various seedhead insects was recorded.

Table 6 shows the results of the dissections. Some of the samples sites surveyed supported high infestations of B. orientalis, U. sirunaseva, and C. hexachaeta. Only a few heads attacked by Eustenopus were found at two locations.

The last survey trip in 1985 was made to the islands of Rhodes and Kos (September 24 - October 1). The purpose of this trip was to locate YST infestations of mature plants so the sites could be revisited in 1986 to search for rosette feeders. On the islands, we looked for YST infestations along the roads driving at a speed of about 30km/h. We made frequent stops and searched the areas by foot.

YST is widely scattered over parts of Rhodes,and in Kos the infestations are much more frequest, except for the mountainous area. The enclosed map shows the areas surveyed, infested areas and the relative density of YST in areas surveyed. To make sure that the plants are in fact C. solstitialis and not C. idaea (as was the case on Crete), herbarium specimens have been sent to Professor Wagenitz (Göttingen, Germany) for identification.

Five seedhead samples were collected from Rhodes and four from Kos. The samples are being held and will be examined for seedhead insects. At each collection site, 25 roots were examined for the presence of root feeders, but none were found. Diseased YST plants were not found and no Centaurea diuffusa or C. maculosa plants were found on either islands.

Table 6. Rate of Infestation by various insects in YST seed head samples, northern Greece, 1985.

No.	Location	No. of heads	C. hexachaeta (different stages) % infestation	U. sirunaseva (galls) % infestation	B. orientalis (eggs) % infestation	Eustenopus (oviposition sites) % infestation
1	Patrida nr. Naussa	100	0	0	0	0
2	Geroplatanos (Arnea)	100	47	3	0	0
3	Charopo (Seres)	100	0	0	5	3
4	Ag. Vasilios	150	32	0	0	0
5	Mesolakia	160	0	22	0.6	0
6	Junction Rodolivos-Angisti	120	0	1	2.5	0
7	Alistrati	130	0	3.8	1.5	0
8	Drama (N. Amisos)	250	3	1.2	5	0
9	Langadas (nr. bridge to Seres)	100	30	1	9	0
10	Kalokastro	100	13	42	38	0
11	Sisamia (city exit)	100	0	0	12	2
12	Milia-Kivotos	100	9	3	3	0
13	20 km W. of Kozani	100	10	1	0	0
14	15 km E. of Peristeroma	140	17	0	0	0

Centaurea diffusa project (U.S. biotypes of C. diffusa)

Seeds of Centaurea diffusa from Spokane, Washington (WA) and Gilliam County, Oregon (OR) were provided by the USDA-ARS Biological Control of Weeds Laboratory at Albany, CA. Each biotype of these seeds was sown in a separate pot on October 15, 1984, and moved out of doors on November 6, 1984 where they were kept under natural conditions in the pots until April 8, 1985 when 32 rosettes of each biotype were transplanted into a field at the Experimental Farm of the University of Thessaloniki. By September 18, sixteen plants of each biotype were still alive and only 6 of the Washington plants and two of the Washington Oregon plants produced flowers in summer 1985, and despite flowering they were still alive on October 8, 1985. In addition, 1,346 rosettes of C. diffusa were collected from the fields near Thermi and transplanted to an experimental plot at the University of Thessaloniki farm. The insects Pterolonche inspersa, Bangasternus provincialis and the eriophyid mite Aceria centaurea were all tested on these North American biotypes of Centaurea diffusa.

Candidate insects:

Pterolonche inspersa is a small moth whose larvae feed on the roots of diffuse knapweed, and it is an important candidate natural enemy for the biological control of diffuse knapweed in North America. In 1985, we set up an experiment with 3 objectives: (1) we wanted to study the possibility of growing a large number of Centaurea diffusa rosettes in a laboratory garden, allow emerging adult P. inspersa moths to oviposit on them in order to collect a large number of infested roots to send to the U.S., from which a large number of adults could be reared out for release.

A second objective was to see if the Pterolonche inspersa moths emerging from infested field collected rosettes which were planted near the large numbers of rosettes transplanted to the experimental plot and the American biotypes would infest the native plants in the experimental plot and accept the American biotypes for oviposition. If the American types were selected for oviposition we wanted to see if they were suitable for development of the insect.

PROCEDURES

On July 14, 1986 110 infested roots of C. diffusa were collected from the fields around Thermi and placed among the rosettes in the experimental plot. Some of these roots were replanted in pots of moist sand which were buried in the ground with the tops at ground level and others were replanted in holes in the ground filled with moist sand.

On July 22, another 80 infested roots were collected near Panorama (hills near Thermi). By this date some of the adults had emerged and eggs could be found on the nearby plants of C. diffusa.

On August 5 four of the infested roots collected on July 14 were examined. Two of the roots each had 1 larva inside, a pupa was found in 1 root and two roots were empty.

On Setpember 13 another 50 of the infested roots collected on July 14 were examined with the following results:

14 roots with pupal exuviae

14 roots with parasitized larvae

3 roots with dead larvae

1 root with a living pupa

18 roots not infested or with larva missing.

This inspection shows that 64% of the plants collected on 14 July were infested and that insects emerged from about 30% of the plants collected.

The C. diffusa plants in the sand filled pots were examined in September. The examination showed that the roots had dried out, and that the larvae made a silken tube ca. 2 cm. long in a downward direction pupated either in the sand outside the root or deep in the root tissue; and never near the crown which was dry. On the other hand the larvae in the plants which had been transplanted in the ground pupated normally near the crown or collar of the plant in late July.

On September 20, 45 roots from those collected July 22 at Thermi were examined. From these we recovered 17 exuviae, 9 parasitized larvae, two dead and one living larvae and 2 dead pupae. The remaining roots were either not infested or the larvae were missing.

On the same day, 25 roots collected on July 14 at Thermi were dissected. From these we recovered 13 pupal exuviae, one parasitized larva, 1 dead larva and 1 dead pupa.

These dissections showed that in mid-September there were some larvae and pupae still alive in the knapweeds.

RESULTS

The results of transplanting the 1,346 rosettes was that 650 were still alive on 18 September 1985 and Pterolonche eggs were present on the rosettes. The yield was not as high as expected, 3 but the final results will be known after the 1986 dissection. With that information in hand we can decide if this is a valid method of producing large numbers of insects for collection and sending for release, provided its release is approved.

It was also learned that there are two specis of parasites which exploit the P. inspersa larvae near Thermi. One is a tachinid fly which is mostly emerged by mid-September and the second is a wasp that is in the pupal stage in mid-September.

Pterolonche inspersa

Objective: To see if P. inspersa moths emerging from indigenous Centaurea diffusa (Greek) would oviposit on US varieties of C. diffusa in an open field situation without cages.

PROCEDURE

On April 8 rosettes of Centaurea diffusa from Oregon and rosettes from Washington (32 rosettes from each location) were transplanted from pots into a small experimental plot on the University of Thessaloniki farm, about 4 m. from the large "garden" of C. diffusa collected locally for Pterolonche production. About 20 infested roots of local C. diffusa were transplanted among these US biotypes on July 14. The plants were left undisturbed and the moths were allowed to emerge and oviposit on the plants to which they were attracted.

RESULTS

On August 25 the plot with the U.S. biotypes of C. diffusa was examined and 17 eggs were found on 10 of the U.S. C. diffusa rosettes and only 10 eggs were found on the 20 Greek rosettes.

The larger number of eggs on the U.S. biotypes does not necessarily indicate a preference for this plant biotype because the U.S. plants were larger and more vigorous, having been transplanted in the plot at least a month earlier than the Greek control plants. The infested rosettes were left in the ground to see if the larvae will mature in the US biotypes of C. diffusa.

Bangasternus fausti

Objective: To see if Centaurea seedhead weevil Bangasternus fausti would accept U.S. biotypes of diffuse knapweed for oviposition and larval development in an open field situation, with no cages.

PROCEDURE

On June 12, 1985, 39 locally collected (Thermi, Greece) adults of Bangasternus fausti were released in an experimental area where 40 local C. diffusa and U.S. biotypes (Oregon and Washington) of C. diffusa were growing. All plants had buds suitable for oviposition. From July 8-August 21 all the mature U.S. C. diffusa flowerheads in the plot were collected weekly, and an equal number of seedheads (n = 1760) was collected from the local plants as was collected from the more abundant Oregon plants.

The samples of heads were kept in cardboard boxes in the laboratory and all the heads were examined from August 26-31 and the number of eggs found on the heads as well as the number of adults dissected from the heads were counted and recorded. The results are presented in Table 7.

RESULTS

On June 13 and 14 many eggs of B. fausti were noticed on both local and US plant biotypes. In addition adults of B. fausti and Larinus minutus (another candidate weevil for diffuse knapweed control) can complete development on U.S. biotypes of diffuse knapweed in an uncaged, open field situation. In the final count there was a higher percentage of eggs on the local and Oregon plants (4%) than on the Washington (2%) plants. The important information provided by this trial is that B. fausti can survive to adult on at least 1 U.S. biotype of diffuse knapweed.

Table 7. Capitula of Greek and U.S. diffuse knapweed plants infested with *Bangasternus fausti* in field conditions.

Plant origin	Seedheads Examined	No. eggs Found	% Infestation	No. adults Emerged	No. adults in Heads
Local (Thermi)	1,760	74	4%	1	1
Oregon	1,760	76	4%	2	1
Washington	375	7	2%	0	0

Aceria centaureae (Nal.) (probably)

In the fall of 1984 an eriophyid mite was found on C. diffusa at Geroplatanos near Arnea. The mite feeds on the rosettes of the weed causing a severe galling, therefore it is considered as a potential candidate for introduction as a biological control agent. Specimens of the mite have been sent to Dott.ssa Marisa Castagnoli, Istituto Sperimentale della Zoologia Agraria, Florence, for determination. The material was not ideal so she gave a tentative identification as Aceria centaureae (Nal.) probably.

Objective: We wanted to determine if this mite would attack US biotypes of diffuse knapweed, to see if we could start a colony in the laboratory garden at Thermi in order to learn something about the mite's biology and how to manipulate it for further tests.

PROCEDURE

In March 1985 the known infestation site at Geroplatanos was visited. No new galls were found, only the dry galls from the preceding year. Examination of the plants showed that the mites overwinter near the root neck of the rosette. Whole rosettes of the field collected plants and dried rosettes broken into pieces were placed on U.S. biotypes and local plants (controls) of C. diffusa which were still in the pots in which they had been grown. Herbarium specimens have been made of infested plants with galls and other mites were collected for identification.

RESULTS

After 8 days, galls were found on both local plant controls and US biotype test plants. The U.S. plants were more heavily galled, perhaps because they were in better condition. The potted rosettes were transplanted into the garden at Thermi and the mites remained on the plants throughout the growing

season. Since the mites prefer the meristem tissue, they galled rosettes as small as the 5 leaf stage. As plant growth slowed the number of galls per plant declined as well as the number of mites per plant.

Dissection of several of the galls showed the mites to be mainly in the galls in the spongy tissue under the epidermis of the leaf. This location keeps the tiny mites from dessicating during the hot dry Greek summers as well as affording them an abundant food supply.

Centaurea diffusa Project

Bionomics of Candidate Insects

Bangasternus fausti

Since this weevil is considered to be a candidate biological control agent for introduction into North America, it is important to have information on its bionomics and behavior, therefore additional observations were made this year and more information was gathered on the insect's distribution in northern Greece, its natural host-range and bionomics.

Distribution in Northern Greece: Bangasternus fausti has been found mainly around Thessaloniki and Seres and to a lesser extent near Kilikis (see map). In western Greece Centaurea diffusa and maculosa were not observed near the roads we surveyed, consequently, no insects were found except for a small population at Kozani.

Host range: In addition to the Centaurea diffusa, C. scabiosa, C. nigra and C. paniculata which are known hosts, we also found the insect attacking C. maculosa as well, in northern Greece.

The first adults of the season were found on C. diffusa on May 7. The same area had been surveyed on April 27, 29 and May 5 but no B. fausti adults were seen on those dates. The first copulating adults were seen May 12 and teneral adults of the new generation were found on July 18 in mature seedheads. In the laboratory the first adults of the new generation emerged on July 18 from a seedhead sample collected July 8. Five of these adults were caged with diffuse knapweed bouquets to observe their activity. They fed a little, moved around very little and finally in late August hid in some paper towels in the bottom of the cage and remained there until October 15 (last observation).

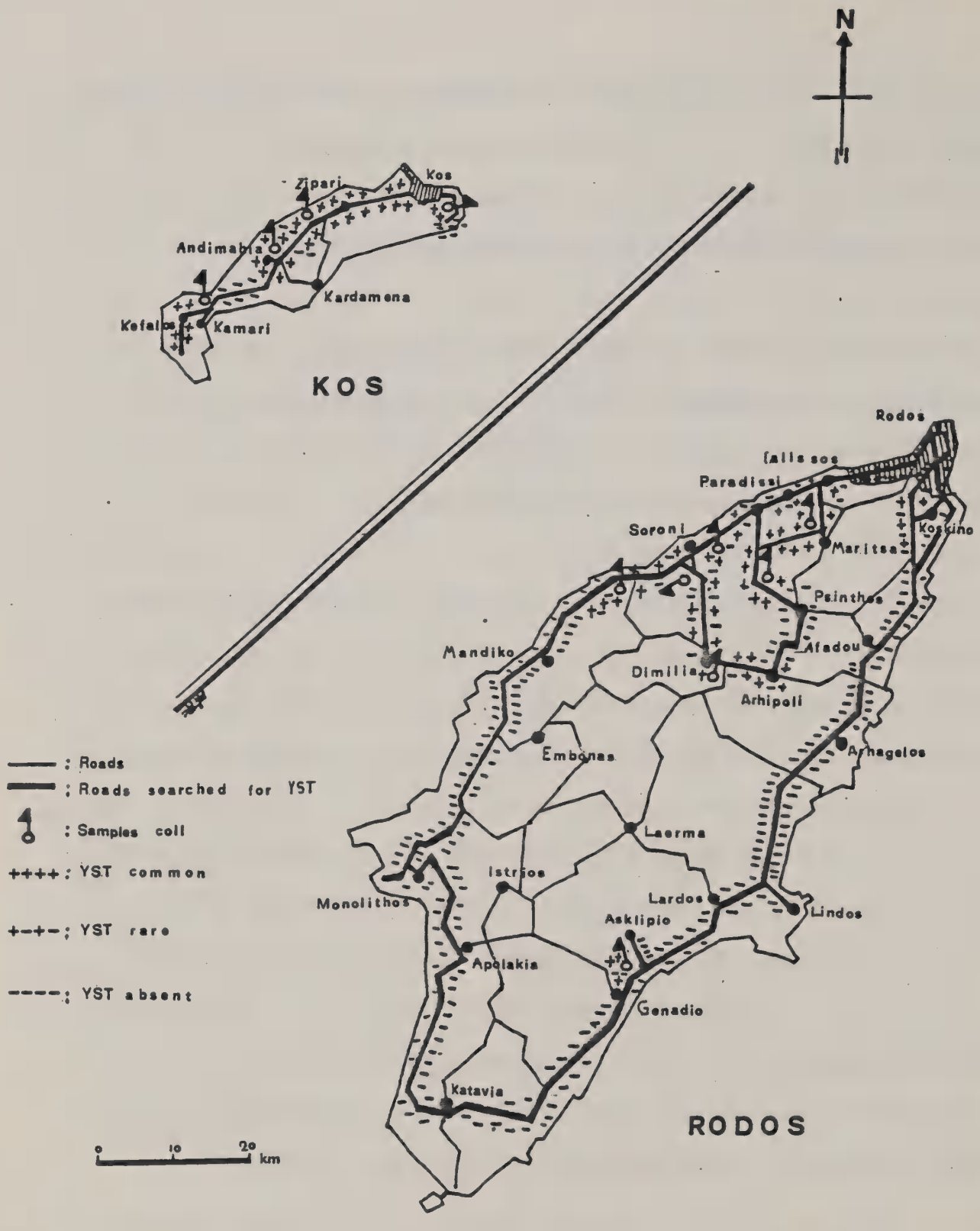
Oviposition behavior: The ovipositional behavior of a single female C. fausti was observed on Centaurea maculosa plant near Triadi, Greece on June 16. She was seen to move from one branch to the other, seeming to search for a proper bud for oviposition. After searching for about 30 minutes she selected a bud 4 mm. long by 2 mm. diameter and examined it for about 5 minutes. Finally with her mandibles she removed the light pubescence from the site she had chosen to deposit an egg. She may have consumed some of the hairs, the others were cut off and pushed aside leaving a smooth surface for her to oviposit on. She assumed an ovipositional position, remaining still for about 3 minutes until the egg was deposited along with a dark green mucous material which covered the egg. Within 5 minutes hardened into a blackish egg cover. After oviposition the female walked upward trailing the mucous behind her, leaving a sort of tail on the egg covering. Usually the tail on the B. fausti egg caps is directed upward while the tail on Bangasternus orientalis egg cap are generally directed downward. After depositing the egg, and walking away making the tail on the egg covering the female for two

minutes, then left the bud walking down to the petiole, from which she flew to an adjacent branch about 10 cm. away. She explored this branch for a few minutes then flew to a dried plant about 20 cms away and from there she flew to the next C. maculosa plant about 1 meter away and we stopped the observations.

Oviposition site selection differs between Centaurea maculata (which has longer petioles) and C. diffusa. Of the 50 eggs counted on C. maculosa, 42 (84%) had been placed on the petioles and 8 (16%) on the leaflets adjacent to the buds. Of the 140 eggs counted on C. diffusa 66 (47%) were laid on the petioles, 73 (52%) on the leaflets and one egg was laid on a bud.

As a general rule the eggs are well distributed with usually only 1 egg per branch tip, but occasionally 2 or 3 eggs were found per branch tip in field collected knapweed samples of both C. diffusa and C. maculosa.

Mortality factors: The egg and larval mortality of B. fausti is quite high. In an aggregate seedhead sample collected on July 12 and August 6, 264 eggs were found. Of these eggs 118 did not hatch, and of the 146 eggs which hatched 85 larvae were found dead in the first instar in the seed heads in the stems or on the petioles. The 204 dead eggs and first instar represent 81% mortality based on the 264 eggs we found. We have the general impression that other mortality factors affect the later stage larvae and that 2% of the eggs deposited produce adult weevils. Some larval parasite have been reared out but no egg parasites have been reared todate, even though the egg parasitism is high.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PAPERS PUBLISHED AND MEETINGS ATTENDED

1. P. Pecora. 14th Congress of Entomology, Palermo. Poster: The Gall Midge Complex on Euphorbia spp., P. Pecora and M. Solinas

2. P. H. Dunn. CSRS Meeting Biological Control of Selected Weed Pests. Pullman, Washington, September 24-25

3. S. L. Clement. National Meeting Entomological Society of America, Hollywood, Florida, December 8-12. Paper "Insect Herbivory on Populations of Yellow Starthistle: Results from a Field Experiment in Italy, S.L. Clement and T. Mimmocchi

4. P. H. Dunn and P. Pecora. Meeting Italian Working Group on Biological Control of Weeds, Piacenza, May 7.

TRAVEL (Rome Laboratory)

February 2	Clement, Sobhian, Dunn, Campobasso, Pecora to Pisa, Annual Meeting USDA/CIBC/CSIRO laboratories.
April 3-4	Pecora, Cristofaro to Pisa, Leafy spurge field work.
April 9-10	Cristofaro, Stazi to Pisa, Leafy spurge field work.
April 9-11	Clement, Mimmocchi to Bari, Santa Severa, Yellow Starthistle field work
April 15-19	Canpobasso to Bari, Centaurea field work
April 22-23	Cristofaro, Stazi to Pisa, Leafy spurge field work
May 6-7	Clement, Mimmocchi to Bari, Yellow Starthistle field work
May 6-8	Dunn, Pecora to Milano-Piacenza, Meeting of Italian Working Group for Biological Control of Weeds at Piacenza; consult with Dott. Leonardi, Milano.
May 8-10	Cristofaro, Stazi to Pisa, Leafy spurge field work.
May 13-28	Campobasso to Thessaloniki, Greece, Diffuse knapweed field work
May 28-29	Clement, Mimmocchi to Castel del Monte, Yellow Starthistle field work
May 30-June 2	Pecora to Palermo, Italian Congress of Entomology
May 30-31	Cristofaro, Stazi to Pisa, Leafy spurge field work
June 12-14	Cristofaro, Stazi to Pisa, Leafy spurge field work
June 19-27	Pecora, Cristofaro to Budapest, Szolnok, Debrecen, Hungary, Vienna, Austria. Leafy spurge collection trip.
June 20-28	Clement to Thessaloniki, Greece, Yellow Starthistle field work
July 2-3	Stazi to Pisa, Leafy spurge field work

July 8-9	Cristofaro, Stazi to Pisa, Leafy spurge field work
July 11-16	Pecora, Cristofaro, Vienna, Budapest, Debrecen, Vienna, Leafy spurge field work
July 13-19	Mimmocchi to Thessaloniki, Greece, Yellow Starthistle field work
July 15-19	Campobasso to Sila, Calabria, Musk thistle field work
July 15-17	Sobhian to Greek Islands, Yellow Starthistle field work
July 21-26	Dunn to Vienna, Thessaloniki, Metsovon, Greece. Administrative matters and YST, diffuse knapweed field work.
July 25-26	Stazi to Pisa, Leafy Spurge field work
September 16-October 24	Dunn to Beltsville, Md., Seattle and Pullman, WA, Salem, OR, Berkeley, CA, Denver, CO, on Home Leave and consultation with U.S. counterparts
September 24-October 30	Sobhian to Piraeus, Kos, Piraeus, Naphlion, Yellow Starthistle and Knapweed survey and collection
October 9-10	Cristofaro, Stazi to Pisa, Leafy spurge field work
October 13-23	Pecora, Cristofaro to Bucharest, Braila (Romania), Vienna (Austria), Leafy Spurge insect collection and field work.
October 29-30	Cristofaro, Stazi to Pisa, Leafy spurge field work
November 12-16	Sobhian to Vienna/Rome/Vienna, return car to Rome, and consult with Rome co-workers.

INSECT AND PATHOGEN SHIPMENTS

ROME LABORATORY

ORGANISM	TARGET WEED	NUMBER AND STAGE	DATE	DESTINATION
Urophora cardui	Canada thistle	1,150 galls	4/1/85	Albany, CA.
Bayeria capitigena	Leafy spurge	150 galls	5/13/85	Albany, CA.
Uromyces scutellatus	Leafy spurge	infested leaves	5/13/85	Ft. Dietrich, Md.
Sphenoptera jugoslavica	Diffuse knapweed	1,000 infested roots	5/21/85	Albany, CA.
Oberea erythrocephala	Leafy spurge	6 adults	6/3/85	Albany, CA.
Bayeria capitigena	Leafy spurge	200 galls	6/3/85	Albany, CA.
Hyles euphorbiae	Leafy spurge	35 pupae	6/11/85	Albany, CA.
Oberea erythrocephala	Leafy spurge	116 adults	6/14/85	Albany, CA.
Bayeria capitigena	Leafy spurge	230 galls	6/17/85	Albany, CA.
Aphthona flava	Leafy spurge	240 adults	6/17/85	Albany, CA.
Bayeria capitigena	Leafy spurge	500 galls (CA)	6/22/85	Albany, CA.
Oberea erythrocephala	Leafy spurge	36 adults	6/22/85	Albany, CA.
Aphthona cyparissiae	Leafy spurge	200 galls (CA)	7/2/85	Albany, CA.
Aphthona flava	Leafy spurge	1,200 adults	7/10/85	Albany, CA.
Bayeria capitigena	Leafy spurge	700 galls (CA)	7/17/85	Albany, CA.
Aphthona cyparissiae	Leafy spurge	120 adults	7/17/85	Albany, CA.
Hyles euphorbiae	Leafy spurge	59 pupae + 7 larvae	7/23/85	Albany, CA.

VISITORS
(in order of visit)

ROME LABORATORY

1. Dr. Roger LeBrun, on sabbatical at the EPL, Béhoust, France, from the Boyce Thompson Institute, Cornell University.
2. Dr. B. D. Perkins, Location Leader, EPL, Béhoust, France
3. Professor Jerzy and Mrs. Isabele Lipa, Poznan, Poland
4. Dr. Mary Carter, Associate Administrator, ARS-USDA, Washington D.C.
5. Mr. Antonio Gayoso, Department of State, Washington D.C.
6. Dr. James E. Ross, FODAG, Rome
7. Mr. James Rudbeck, Agricultural Counselor, American Embassy, Rome
8. Ms. Mariella Mecozzi, Agriculture, American Embassy, Rome
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10. Dott.ssa Laura Picciafuoco, Università La Sapienza, Rome
11. Dr. Charles Turner, USDA-ARS-BCWL, Albany, CA.
12. Dr. Larry Littlefield, Head, Department Plant Pathology, Oklahoma State University
13. Dr. B. D. Perkins, Location Leader, EPL, Béhoust, France
14. Dr. Martin Birch, Zoology Department, Oxford University, England
15. Dr. Jack DeLoach, USDA-ARS, Temple, TX
16. Dr. Charles Turner, USDA-ARS-BCWL, Albany, CA.
17. Dr. T.B. Kinney, Administratory, ARS-USDA, Washington D.C.
18. Dr. Charles Obrien, Florida A&M University, Tallahassee, FL.
19. Dr. J.E. Wright, NPS-ARS-USDA, Beltsville, Md.
20. Dr. Susanna Mariani and Dr. Giovanni Vita, Laboratory of Technology for Defense of Agrosystems, Rome
21. Dr. Sister Mary Frances Traynor and Sister Nancy Hutchinson, Franciscan Sisters of the Eucharist, Assissi, Italy
22. Dr. Max Whitten, Division of Entomology, CSIRO, Canberra, Australia.

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Defago, G., Zurich, Switzerland

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Division of Biocontrol, Dept. of Entomology, UCR Riverside, CA.

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Agricultural Counselor, American Embassy, Athens, Greece

S. L. Clement, Plant Germplasm Research, USDA, ARS,Pullman, Washington

NAL, Beltsville, Md.